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REVUE MENSUELLE DES SCIENCES PURES ET APPLIQUÉES
MONATSSCHRIFT FÜR DAS GESAMTE GEBIET DER NATURWISSENSCHAFT
RIVISTA MENSILE DI SCIENZE PURE E APPLICATE
MONTHLY JOURNAL OF PURE AND APPLIED SCIENCE

Editores:

P. HUBER · R. MATTHEY · A. v. MURALT · L. RUZICKA
Basel Lausanne Bern Zürich

Redactor: H. MISLIN, Basel-Mainz

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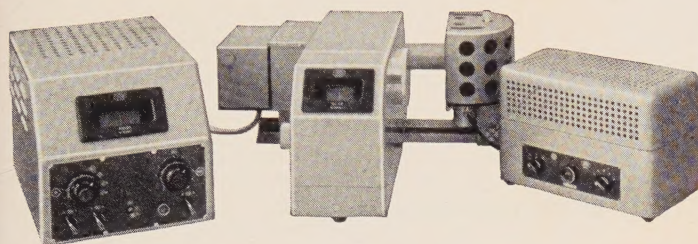
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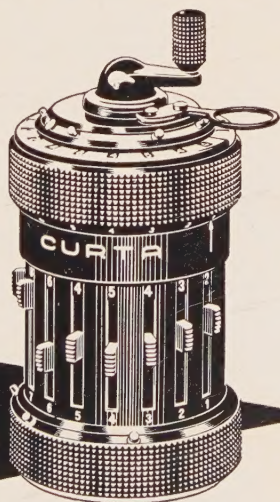
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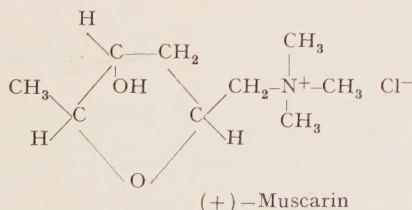
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Über Muscarin¹

Synthese eines Gemischs von Muscarin und seinen stereoisomeren Formen

In einer Untersuchung² von KÖGL, SALEMINK, SCHOUTEN und JELLINEK wurde über neue Ergebnisse auf dem Muscaringebiet berichtet. Die im Jahre 1931 für Muscarin angenommene Konstitution eines quaternären Trimethylammoniumsalzes von α -Amino- β -oxyvaleraldehyd $[C_8H_{15}O_2N]^+Cl^-$ hat sich als unrichtig erwiesen. Die nunmehr mit Hilfe chromatographischer Methoden ausgeführte Isolierung dieses Alkaloids aus Fliegenpilzen führte in Übereinstimmung mit neueren Untersuchungen aus anderen Laboratorien (EUGSTER³, BALENOVIC⁴ *et al.*, KUEHL *et al.*⁵) zur Bruttoformel $[C_9H_{20}O_2N]^+Cl^-$. Wir fanden, dass Fliegenpilze daneben auch das beim pharmakologischen Test am Froschherzen noch aktivere *Acetylcholin* enthalten. Beim Hofmann'schen Abbau des Muscarins konnte ausser Normuscarin nur Trimethylamin als Abbauprodukt identifiziert werden. Die beiden Sauerstoffatome liegen in einer Hydroxylgruppe und einem Tetrahydrofuranring vor. Letzteres ergab sich unter anderem aus dem Infrarot-Absorptionsspektrum. Durch Reduktion des Muscarins mit Jodwasserstoffsäure und nachfolgende Hydrierung liess sich Trimethyl-hexyl-ammoniumhydroxyd in Form des Chloroaurats isolieren. Diese Base enthält - abgesehen von den beiden Sauerstoffatomen - noch das gesamte Skelett des Muscarin-Kations. Ihre Bildung steht nicht im Einklang mit den von EUGSTER³ angenommenen Formeln für Muscarin. Die endgültige Festlegung der Struktur und des räumlichen Baus erfolgte durch die Röntgenanalyse⁶. Muscarin ist das quaternäre Trimethyl-ammoniumsalz von 2-Methyl-3-oxy-5-(amino-methyl)-tetrahydrofuran:



In Anbetracht der drei asymmetrischen Kohlenstoffatome sind bei der Synthese *vier* Racemate zu erwarten, falls man von inaktivem Material ausgeht und bei den angewandten Methoden alle theoretisch möglichen Konfigurationen entstehen. Von den verschiedenen Projekten zur Synthese des Muscarins und seiner Stereoisomeren hat das umstehende Reaktionsschema zuerst zu einem Erfolg geführt.

Die Konstitution des als Ausgangsmaterial dienenden α -Acetyl- δ -chlor- γ -valerolactons⁷ (I) haben wir durch Hydrolyse und Perjodat-Titration sichergestellt. Das Lacton wurde mit einem Mol Trimethylamin eine Stunde auf 150° erhitzt. Nach Entfernung des unumgesetzten Trimethylamins und Ausschütteln mit Äther haben wir den wässrigen Auszug des Reaktionsprodukts über Norit chromatographiert. Das quaternäre Trimethylammoniumsalz II bildet farblose Kristalle vom Smp. 228°. Die Chlorierung zu Verbindung III erfolgte unter Eiskühlung durch tropfenweises Zufügen von frisch destilliertem Sulfurylchlorid⁸. Das allmählich erstarrende Reaktionsprodukt wurde einen Tag im Vacuum über Ätznatron bewahrt und dann eine Lösung in 20prozentiger Salzsäure auf 100° erhitzt, bis die Kohlensäureentwicklung beendet war. Nach Neutralisation mit $BaCO_3$ und Eindampfen haben wir den Rückstand zur Entfernung anorganischer Salze mit absolutem Alkohol ausgezogen. Aus diesem Extrakt liess sich das kristallinische Chloroaurat des Stereoisomerengemisches von IV isolieren. Die Reduktion der Ketogruppe von IV erfolgte mit Natriumborhydrid in wässriger Lösung. Zur Entfernung der Borsäure wurde angesäuert und wiederholt mit Methanol abgedampft. Die durch Extraktion mit absolutem Alkohol gewonnene Verbindung V haben wir in konz. H_2SO_4 aufgenommen und diese Lösung 5 min auf 90° erwärmt. Wie REPPE⁹ gefunden hat, erfolgt unter ähnlichen Bedingungen bei verwandten 1,4-Diolen der Ringschluss zum entsprechenden Tetrahydrofuran. In unserem Fall führt der Ringschluss zwar zum erwarteten Chlor-derivat VI, dagegen fanden wir keine geeignete Arbeitsweise für den Austausch des Chloratoms durch die Hydroxylgruppe. Zur Umgehung dieser Schwierigkeit wurde die Lösung von Verbindung V in Eisessig in der üblichen Weise mit Silberacetat behandelt. Nach Entfernung der Silbersalze haben wir Verbindung VII (als Rohprodukt) zur Hydrolyse mit 20prozentiger H_2SO_4 7 h auf 125° erwärmt. Hierauf wurde im Vacuum bei einer Badtemperatur von 40° eingeeengt und die auf diese Weise erhaltene Lösung der Triol-base in konzentrierter H_2SO_4 10 min auf 90° erhitzt. Die übliche Aufarbeitung lieferte einen Sirup, dessen wässrige Lösung wir über Norit chromatogra-

¹ 4. Mitteilung über Muscarin.

² F. KÖGL, C. A. SALEMINK, H. SCHOUTEN und F. JELLINEK, *Rec. trav. chim. Pays-Bas* (im Druck).

³ C. H. EUGSTER, *Helv. chim. Acta* **39**, 1002, 1023 (1956). - Vgl. auch C. H. EUGSTER und P. G. WASER, *Exper.* **10**, 298 (1954).

⁴ K. BALENOVIC, D. CERAR, B. GASPERT und T. GALIJAN, *Arkiv Kem.* **27**, 107 (1955).

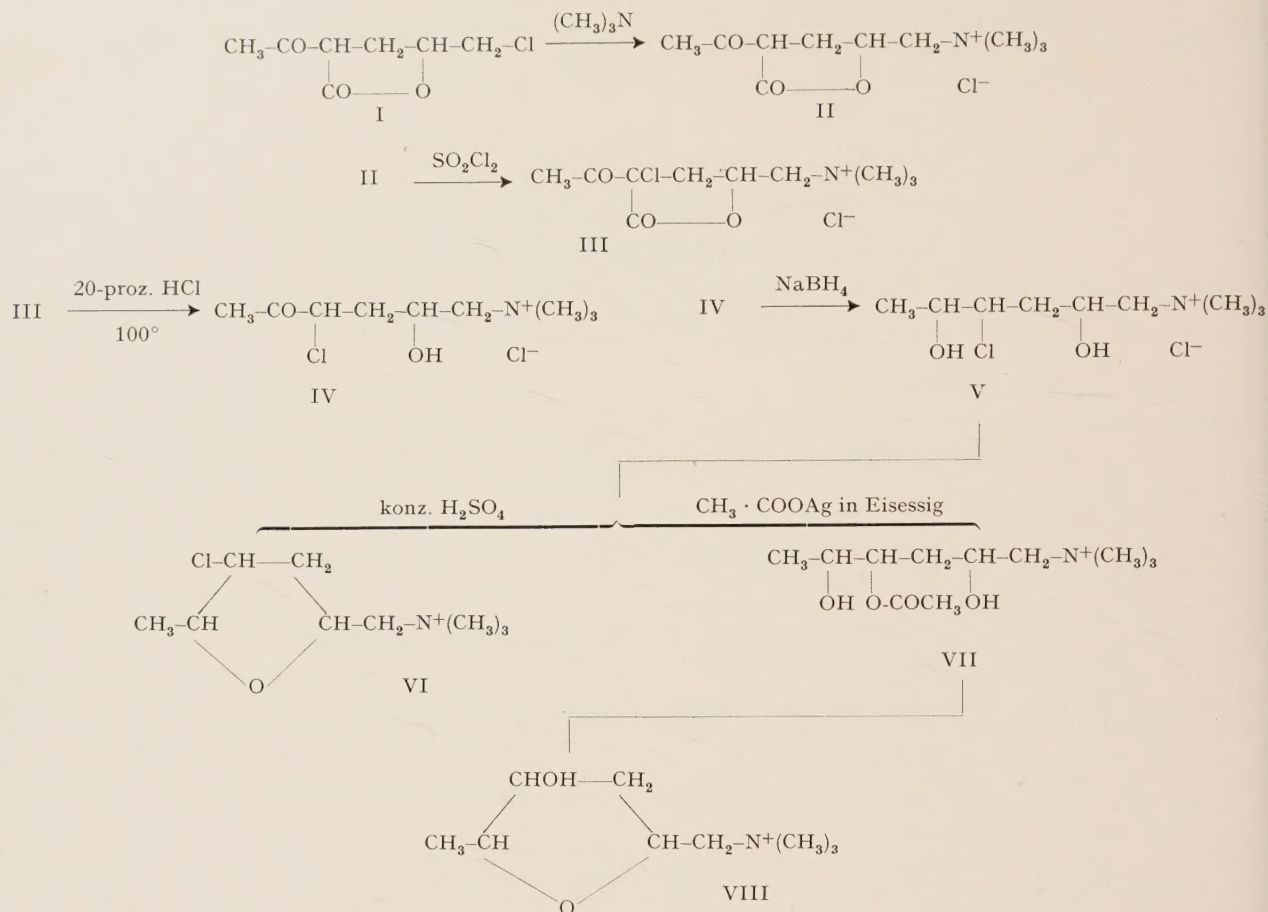
⁵ F. A. KUEHL, N. LEBEL und J. W. RICHTER, *J. Amer. chem. Soc.* **77**, 6665 (1955).

⁶ Laboratorium für Kristallchemie der Rijksuniversiteit Utrecht (Direktor Prof. Dr. J. M. BIJVOET); siehe F. JELLINEK, *Acta Crystallographica* (im Druck).

⁷ W. TRAUBE und E. LEHMANN, *Ber.* **34**, 1971 (1901).

⁸ Vgl. u. a. J. R. STEVENS und G. A. STEIN, *J. Amer. chem. Soc.* **62**, 1045 (1940).

⁹ W. REPPE, *Liebigs Ann. Chem.* **596**, 90, 118 (1955).



phierten. Aus den Mittelfractionen liess sich ein kristallisiertes Chloroaurat isolieren, das nach Umkristallisieren aus Wasser bei 69–72° schmolz.

$\text{C}_9\text{H}_{20}\text{O}_2\text{NAuCl}_4$ (513)

Ber. C	21,24	H	3,90	N	2,73	Cl	27,68	Au	38,40
Gef.	21,34		3,94		2,77		27,92		38,67

Das durch die Zerlegung des Chloroaurats erhaltene Chlorid zeigt papierchromatographisch einen R_F -Wert von 0,50 in Butanol-Äthanol-Wasser (5:5:2), übereinstimmend mit dem R_F -Wert von natürlichem Muscarin. Die Infrarot-Spektren des synthetischen Produkts und des natürlichen Alkaloids stimmen in den Hauptbanden weitgehend überein, nur in einigen Gebieten (zum Beispiel bei CH-Frequenzen) zeigt ersteres viel weniger ausgeprägte Feinstrukturen, wie das bei einem Gemisch von Stereoisomeren erwartet werden kann.

Die pharmakologische Wirksamkeit am Froschherzen beträgt bei dem synthetischen Gemisch von Stereoisomeren etwa ein Drittel von der des Naturprodukts.

F. KÖGL, H. C. COX und
C. A. SALEMINK

Organisch-chemisches Laboratorium der Rijksuniversiteit Utrecht (Holland), den 28. Februar 1957.

Summary

A recent investigation of KÖGL, SALEMINK, SCHOUTEN, and JELLINEK established the constitution of muscarin as the quaternary trimethyl-ammonium salt of 2-

methyl-3-hydroxy-5-(amino-methyl)-tetrahydrofuran. Moreover the X-ray analysis elucidated the complete stereochemical configuration of the alkaloid.

There are various possibilities for a synthetic approach. The present communication gives a preliminary description of a synthesis which started from α -acetyl- δ -chloro- γ -valerolactone, leading to a mixture of muscarin and its stereochemical isomers. The synthetic product gives the same R_F -value and a very similar infrared spectrum. The pharmacological activity on the frog heart is about one third of that of the natural alkaloid.

Synthese von Stereoisomeren des Muscarins¹

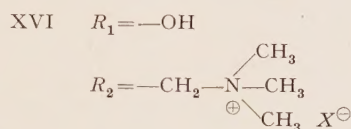
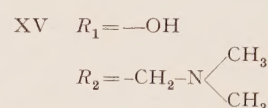
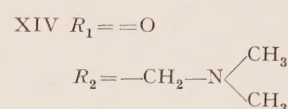
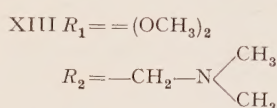
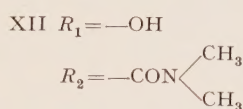
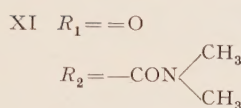
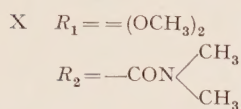
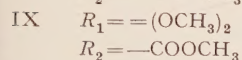
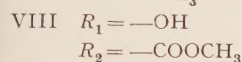
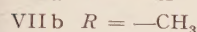
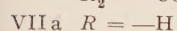
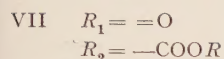
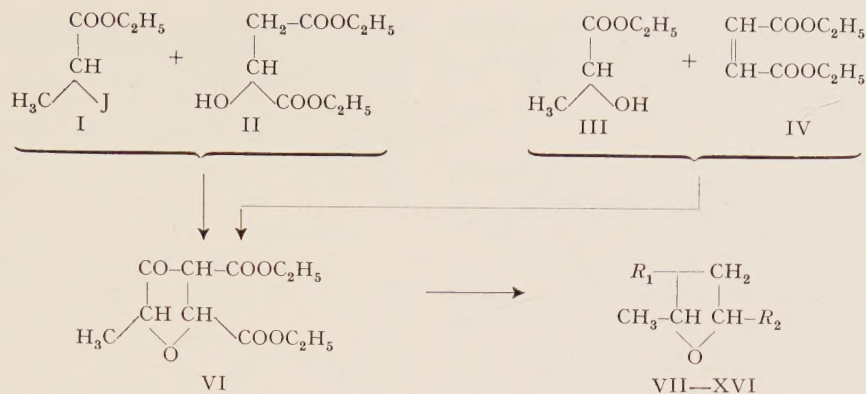
Im Anschluss an die Versuche von F. KÖGL, H. C. COX und C. A. SALEMINK² berichten wir kurz über weitere Synthesen auf dem Muscarin-Gebiet, die gemäss folgendem Schema ausgeführt wurden.

α -Jodpropionsäure-äthylester (I) gab beim Kochen mit Äpfelsäure-diäthylester (II) und Silbercarbonat in Dioxan einen Äther-triester V³ (Kp. 0,1 135°, ν CO bei 1740 cm^{-1}), welcher beim Cyclisieren mit Natrium-äthylat zu dem über das Kupfersalz $\text{C}_{22}\text{H}_{30}\text{O}_{12}\text{Cu}$, Smp. 177–181°, isolierten β -Keto-dicarbonensäureester VI (Kp. 0,1 110–125°, ν CO bei 1780 cm^{-1} , 1740 cm^{-1}) führte.

¹ 5. Mitteilung über Muscarin. Vorläufige Mitteilung.

² Siehe vorangehende 4. Mitteilung über Muscarin.

³ Von allen in dieser Mitteilung erwähnten Verbindungen wurden gutstimmende Analysenwerte erhalten.



VI konnte auch durch Kondensation von Milchsäure-äthylester (III) und Maleinsäure-äthylester (IV) mit Natrium erhalten werden⁴; IR.-Spektrum identisch mit vorigem Produkt. Verseifung des Keto-dicarbonsäureesters VI mit Schwefelsäure lieferte die 2-Methyl-3-tetrahydrofuranon-5-carbonsäure (VIIa), Kp. _{0.01} 105°, die mit Diazomethan zum 2-Methyl-5-carbomethoxytetrahydrofuranon-(3) (VIIb) (Kp. ₁₁ 107°, ν CO 1770 cm⁻¹, Schulter bei 1740 cm⁻¹) verestert wurde.

Der γ -Ketoester VIIb diente als Ausgangsprodukt für verschiedene Synthesen.

Hydrierung von VIIb mit Raney-Nickel gab den Hydroxyester VIII (Kp. _{0.05} 110°, ν OH 3500 cm⁻¹, ν CO 1740 cm⁻¹). Ein Produkt derselben Bruttozusammensetzung wurde auch durch Reduktion von VIIb mit Natriumborhydrid erhalten (Kp. _{0.01} 110°, identisches IR.-Spektrum). Umsetzung des Hydroxyesters VIII mit Dimethylamin im Bombenrohr führte zum Hydroxydimethylamid XII (Kp. _{0.01} 135°, ν OH 3550 cm⁻¹, ν CO 1640 cm⁻¹), aus welchem durch Reduktion mit Lithiumaluminiumhydrid ein Gemisch von Stereoisomeren der Konstitution XV bereitet wurde. (Kp. _{0.01} 100–105°, ν OH 3500 cm⁻¹). XV wurde mit Methyljodid ins teilweise kristallisierte Methojodid XVI (X = J) übergeführt, welches mit AgCl das Gemisch der Stereoisomeren von Muscarinchlorid (XVI, X = Cl) ergab; C₉H₂₀O₂NCl Ber. C 51,54 H 9,61%; Gef. C 51,14 H 9,84%, ν OH 3400 cm⁻¹. IR. praktisch identisch mit natürlichem Muscarinchlorid, ebenso R_F -Werte in 3 verschiedenen Systemen. XVI gab mit Tetraphenylboratnatrium ein schön kristallisiertes Tetraphenylboronat-Gemisch C₃₃H₄₀O₂NB, Smp. 152° unscharf.

Eine weitere Synthese aus VIIb führte über das Dimethylketal IX (Kp. ₁ 121°, ν CO 1740 cm⁻¹) zum Di-

methylketal-dimethylamid X (Kp. _{0.01} 140°, ν CO 1650 cm⁻¹) und weiter zum Ketoamid XI (Kp. _{0.01} 140°, ν CO 1770, 1660 cm⁻¹). Dinitrophenylhydrazon-Gemisch C₁₄H₁₇O₆N₅, Smp. 195°. Reduktion des Ketoamids XI gab wieder ein Gemisch von Stereoisomeren der Formel XV, Kp. _{0.01} 100°.

Reduktion des Dimethylketal-dimethylamids X mit Lithiumaluminiumhydrid ergab über das nichtisolierte Ketal XIII das 2-Methyl-5-dimethylaminomethyl-tetrahydrofuranon-(3) (XIV), Kp. _{0.01} 90°, ν CO 1770 cm⁻¹, aus welchem mit Lithiumaluminiumhydrid ein Gemisch von Stereoisomeren der Formel XV erhalten wurde.

Über die Trennung der Racemate und ihre pharmakologische Wirksamkeit wird später an anderer Stelle berichtet werden.

H. CORRODI, E. HARDEGGER,
F. KÖGL und P. ZELLER

Organisch-chemische Laboratorien der Eidgenössischen Technischen Hochschule in Zürich, der Rijksuniversiteit Utrecht (Holland) und der F. Hoffmann-La Roche & Co. AG., Basel, den 28. Februar 1957.

Summary

Using ethyl α -iodopropionate (I) and diethyl malate (II) or ethyl lactate (III) and diethyl maleate (IV) as starting materials, the furanone derivatives VI and VIIb have been prepared. The latter has been converted into a mixture (XVI) of muscarin and its stereoisomers by various methods summarized in the formulae.

⁴ Vgl. M. DAVIS und W. BRADLEY, Brit. Pat. 568.402 (3.4. 1945).

Membrane Potentials of the Squid Giant Axon Recorded with an Inserted Antimony Microelectrode

Direct measurements of cellular membrane potentials are usually made with KCl-filled microcapillaries in preference to metallic insertion electrodes. The former besides being easy to prepare¹ are not subject to variations in contact potentials which often develop with the latter. Nevertheless, some metallic electrodes have a realm of special usefulness. Thus, a reversible Ag-AgCl electrode can serve to determine the electrochemical activity of Cl^- in an appropriate system. For the squid giant axon it has been shown² that Cl^- inside and outside the excitable membrane is not in thermodynamic equilibrium. This note reports measurements of intra-axonal potentials by means of antimony electrodes which, when properly used, are reversibly sensitive to H^+ .

Glass-insulated Sb microelectrodes³ with overall tip diameters of $5\text{--}10\mu$ were connected to an amplifier which, because of the low magnitude and polarity of its grid current did not affect the H^+ sensitivity of the electrodes. The electrodes were checked before insertion into and after removal from the axon. Against a Ag-AgCl reference, a standard phosphate buffer of pH 7 was 170–180 mV more negative than a buffer of pH 4.

Results. Measurements were obtained on six nerves. The axon was first impaled with a KCl-filled microcapillary. The resting potential (-60 mV) and the spike

(110 mV) recorded in this way are shown in Figure 1. Subsequent insertion of the Sb electrode less than 1 mm from the site of the microcapillary did not affect the potentials at the latter. The initial potential in sea water between the Sb electrode and the reference electrode (considered as the zero potential) changed abruptly and violently in a positive direction when the Sb electrode touched, but did not penetrate the axon. The shift, which varied between 100 and 200 mV, may have been caused by a temporary increase in the resistance as the Sb surface came into contact with lipid material of the axonal sheath. Small potential variations, usually not more than 5 mV, are frequently obtained when a KCl microcapillary is pressed against the axon.

As soon as the Sb electrode penetrated the axonal membrane, however, it registered a value closely approximating the resting potential recorded with the KCl microcapillary. The small difference (4 to 7 mV, Fig. 1 and 2) is ascribable to junctional potentials. Penetration of the Sb electrode into the axon was further signaled by the appearance of transmembrane spikes almost identical with those recorded by the KCl microcapillary.

In two experiments, 0.54 M KCl was added to the sea water surrounding a 1.2 cm stretch of the axon located in a well separated from the rest of the nerve chamber by vaseline seals. Both electrodes impaled the central portion of the axon lying within this well. One end of the axon was stimulated. Externally recording electrodes, straddling the well, monitored the propagation of the impulse into the well and out of it.

Both internal electrodes recorded identically the depolarization and decrease of spike amplitude caused by the addition of KCl (Fig. 1 and 2). The depolarization began immediately and continued to develop while the KCl was again replaced with sea water, since removal of the excess KCl by dilution was a relatively slow process. The response lost is overshoot and propagation

¹ G. LING and R. W. GERARD, *J. cell. comp. Physiol.* **34**, 383 (1949). – W. L. NASTUK and A. L. HODGKIN, *J. cell. comp. Physiol.* **35**, 39 (1950). – C. Y. KAO, *Science* **119**, 846 (1954).

² A. MAURO, *Fed. Proc.* **13**, 96 (1954).

³ We are indebted to Dr. J. Y. LETTVIN, Department of Electrical Engineering, Massachusetts Institute of Technology, for providing the electrodes and for his kind help.

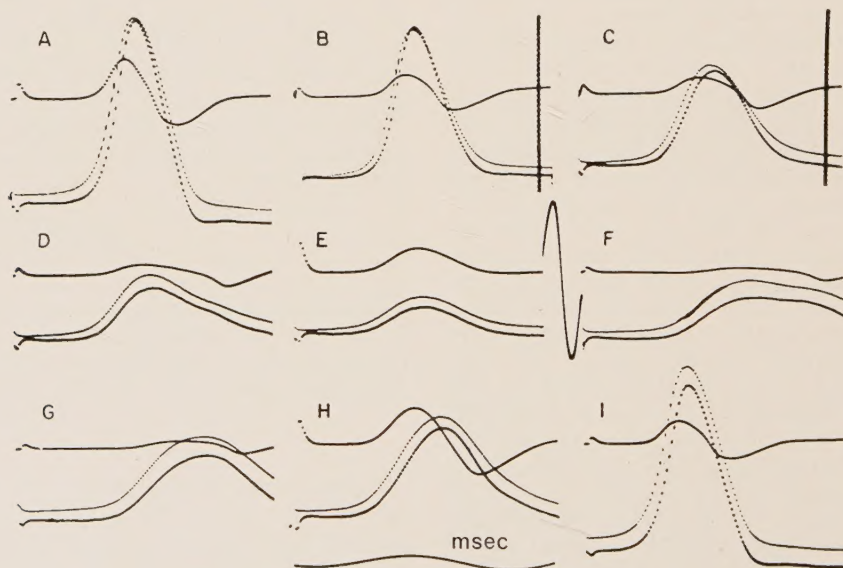


Fig. 1.—Membrane potentials simultaneously recorded with KCl and Sb microelectrodes inserted into loci less than 1 mm apart of squid giant axon. *Upper trace*: Zero potential base line for both microelectrodes prior to impalement; also carries the record of diphasic spike, propagating into and out of impaled region. *Two traces below*: KCl and Sb electrodes, respectively. *A*, initial record. *B*, within first second after adding 0.54 M KCl to impalement region. Signal marker indicates the addition of the KCl. *C*, fifth second. *D*, at 10 s, prior to block of conduction; *E*, at 15 s, maximum block. *F* to *I*, recovery at 25, 30, 35 and 65 s, respectively. Calibration, 100 mV, between *E* and *F*.

ceased when the resting potential in the recording region fell to 40 mV (Fig. 1D, E). Recovery of response height and of conduction (Fig. 1F, G) were associated with a repolarization of only 2 mV (Fig. 2). The spike amplitude increased more rapidly than did the resting potential, except toward the end of recovery, when rapid repolari-

potentials recorded with the different electrodes, is that the resting potential is not due to a Donnan potential resulting from the asymmetrical distribution of ions, but is a consequence of active processes in the membrane.

C. Y. KAO⁷ and H. GRUNDFEST⁸

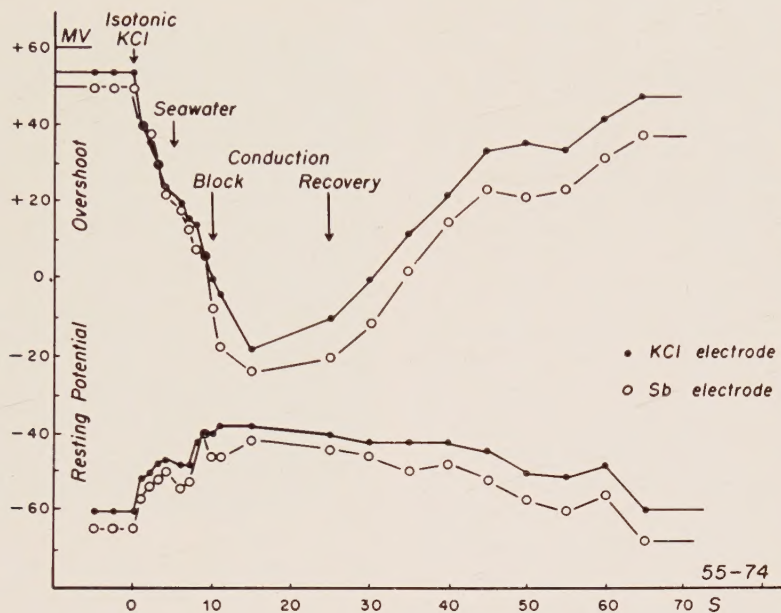


Fig. 2.—Time course of membrane potential changes and character of the response produced by changing KCl concentration of the external medium. Same experiment as in Figure 1.

zation brought both the resting potential and the spike almost fully back to their initial values.

Discussion. If the internal H^+ concentration were determined by a Donnan distribution, a potential of 60 mV, inside negative, recorded with the Sb microelectrode would indicate that the interior of the axon should have a pH of about 9, since that of the sea water was about 8. However, colorimetric determinations disclose an axoplasmic pH of 6.6 and 6.8 for giant axons of *Sepia* and *Loligo* respectively⁴, values which should correspond to a potential of about 70 mV, inside positive. These results are not in agreement with a Donnan distribution even assuming that the potential recorded with the Sb electrode were determined by an algebraic summation of the potential of the H^+ distribution ratio and of the resting potential (of whatever independent origin) as determined by the KCl-filled microcapillary. In crab and frog muscle fibers⁵, the internal pH is likewise different from that calculated on the basis of the Donnan ratios.

Conceivably, in the axoplasmic medium, the Sb electrode failed to serve as a pH electrode, despite the fact that before insertion into and after removal from the axon it recorded H^+ activity reversibly. An alternative interpretation⁶, made more likely by the nearly identical

Department of Physiology and Pharmacology, State University of New York, and Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, December 3, 1956.

Résumé

Les enregistrements pris simultanément des axones géants de *Loligo* au moyen de microélectrodes de KCl et de l'antimoine donnent à peu près les mêmes valeurs pour le potentiel de repos et pour la pointe. Les deux microélectrodes enregistrent d'une façon identique les changements de potentiel de la membrane pendant la dépolarisation avec KCl et pendant la restitution. Bien que les microélectrodes de Sb restent toujours réversibles à H^+ , les potentiels enregistrés ne correspondent pas à la distribution de H^+ . Ces données, comme d'autres, indiquent que le potentiel de repos n'est pas une conséquence des distributions de DONNAN, mais plutôt, selon toute probabilité, un effet des propriétés de la membrane électrogène.

⁷ Present address: Rockefeller Institute for Medical Research, New York.

⁸ This work was carried out at the Marine Biological Laboratory, Woods Hole, Mass., with a grant under MBL contract (Nonr-09705). Additional aid was received from American Philosophical Society, Muscular Dystrophy Associations of America, National Science Foundation and United Cerebral Palsy Associations.

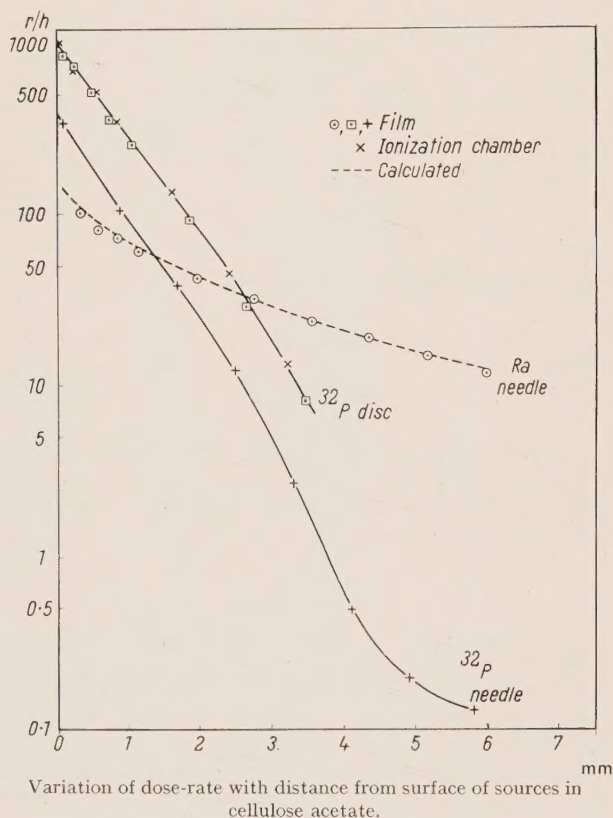
⁴ A. ARVANITAKI and M. CHALAZONITIS, Arch. Sci. Physiol. 5, 207 (1951). — R. CHAMBERS and C. Y. KAO, Exp. Cell. Res. 3, 584 (1952).

⁵ P. C. CALDWELL, J. Physiol. 127, 169 (1954). — A. V. HILL, Proc. Roy. Soc. B 144, 1 (1955).

⁶ H. GRUNDFEST, C. Y. KAO, and M. ALTAMIRANO, J. gen. Physiol. 38, 245 (1954). — H. GRUNDFEST in *Electrochemistry in Biology and Medicine* (T. SHEDLOVSKY, editor, Wiley, New York 1955). — A. L. HODGKIN and R. D. KEYNES, J. Physiol. 131, 502 (1956).

Dose Measurements with Photographic Films on a Beta-Ray Needle

The clinical use of thin-walled tubes containing a beta-ray emitter has already been reported¹, and it is evidently of some importance to know the dose-rates delivered by such tubes at the surface and at a depth in tissue. Because of the source geometry, the measurement of these dose-rates with ionization chambers is difficult, and a film method such as has already been used on plane beta-ray sources², is more likely to meet with success. The purpose of this note, reporting the results of some measurements made with films on a beta-ray needle loaded with phosphorus 32, is to demonstrate that films may be used with a reasonable degree of accuracy to measure the dose-rates from beta-ray tubes and needles.



The needle was a hollow duralumin tube (external diameter 2 mm; wall thickness 0.2 mm), closed with a point at one end, and having a tight fitting plug at the other. The internal space, 2.5 cm long, was filled with phosphorus 32 solution. The film used for the measurements was Ilford Industrial F, the suitability of this film for dosimetry having already been demonstrated³. Each film was exposed separately in a plane parallel to that of the needle, with different thicknesses of cellulose acetate absorbers (density 1.32 g/cm³) in between. Films were also exposed similarly to a 1 mg radium needle

(active length 15 mm; external diameter 1.85 mm; filtration 0.6 mm Pt.), and to a disc of phosphorus 32 plastic, 22 mm diameter and 0.5 mm thick. Each batch of films was developed in Ilford ID 19 developer together with a series of films that had received standard gamma-ray exposures from a 25 mg radium tube. For these standard exposures, the films were sandwiched between layers of Perspex 6 mm thick, and set up in a Perspex jig at distances varying from 14.5 to 65 cm from the tube for a period of about 16 h. By this means a series of known exposures from 0.8 to 16 r were obtained. After processing, the films were densitometered with an EEL Universal Minor Densitometer using a 1 mm spot size. Readings of density were then converted to dose in roentgens by comparison with the films that had received known doses from the 25 mg radium tube.

The results are shown in the Figure. The estimated dose-rates on the phosphorus 32 plastic were in good agreement with values measured on the same disc with a shallow thin-windowed ionization chamber having a collecting volume 1 cm in diameter by 1 mm deep. The validity of the film technique for beta-ray dosimetry was thus confirmed. Film measurements on the 1 mg radium needle agreed well with values calculated using SIEVERT's integrals taking into account oblique filtration. This agreement supported the view that the film method might be especially suitable for the dosimetry of line sources. Finally, the measurements on the beta-ray needle, made when its activity was about 160 μ c, showed very clearly the limitations of this form of therapy for other than very superficial conditions. By comparison with the radium needle, the dose fell off extremely rapidly with depth, and, as was to be expected from geometrical consideration, the half-value depth (0.45 mm of absorber, equivalent to 0.56 mm of tissue), was a little less than that for the disc-shaped beta-ray source.

We are grateful to Professor J. S. MITCHELL for his interest and advice.

J. L. HAYBITTLE and GIOVANNA MAYR*

Department of Radiotherapeutics, University of Cambridge, December 4, 1956.

Zusammenfassung

Es werden Messungsergebnisse mitgeteilt, die zeigen, dass die photographische Filmtechnik geeignet ist, Dosismessungen an linearen Betastrahlern (Nadeln oder Tuben) vorzunehmen.

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A Microradiographic Study of Bone Tissue in Ovarian Dermoid Cyst

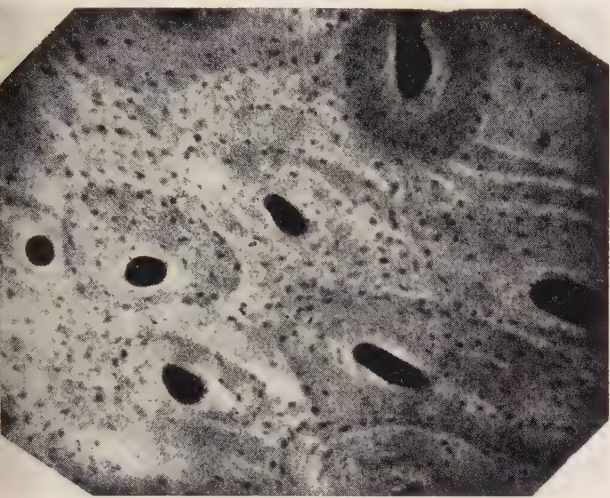
Dermoid cyst is a teratoma and one of the common tumors of the ovary. The cyst has a yellow colour, a doughy consistency and its wall is lined with a cubical epithelium. The contents of an ovarian dermoid show many variations. Mostly it is a yellowish, turbid, oily material containing hair and skin (hence the name dermoid). One often sees bone, teeth, cartilage, thyroid, brain, intestine, striated muscle, adrenal, etc.

¹ F. CRAINZ, *Radioisotope Conference 1954* (Butterworths, London 1954), p. 11. – A. THULLEN, *Strahlentherapie*, Supplement 35, 129 (1956).

² E. TOCHILIN and R. GOLDEN, *Nucleonics* 11 (8), 26 (1953).

³ J. L. HAYBITTLE, *Brit. J. Rad.* (in press).

The origin of an ovarian dermoid is supposed by some authors to be one of the ova of the ovary. Another theory supposes the dermoid cyst to arise from one of the original blastomeres formed by the primary segmentation of the ovum, which has become separated and included in the ovary¹.



Bone tissue in an ovarian dermoid cyst has been studied by a microradiographic technique. Sections of about 30μ have been exposed to X-rays of the wavelength $2.4-4 \text{ \AA}$. (A Machlett tube AEG 50 with 1 mm Be filter, has been used.) Within these limits calcium has one of its absorption edges (K-edge at $3.06 \text{ \AA}$). Other components of bone show here a very slight absorption. Because of this the microradiogram shows the calcium distribution in the specimen. (Film used: Kodak Spectroscopic Plate 649.)

The figure demonstrates that there is well differentiated bone tissue in the dermoid cyst. Compact regions with Haversian systems of different ages are to be seen. Systems which are old show a higher degree of mineralization (light in figure) than those which are young (darker in figure). Regions also appear with a more spongy structure. The figure shows a microradiogram of bone tissue in an ovarian dermoid cyst from a woman of 40 years of age.

H. RÖCKERT

Department of Histology, University of Göteborg, Sweden, December 20, 1956.

Zusammenfassung

Die Verteilung von Kalzium im Knochengewebe in ovarialen Dermoidzysten wurde mit Hilfe der mikroradiographischen Technik studiert. Dabei konnten Havers'sche Systeme in verschiedenen Mineralisierungsstadien, die eine gut differenzierte Entwicklung des Knochengewebes andeuten, gesehen werden.

¹ R. AMPRINO and A. ENGSTRÖM, Acta Anat. 15, 1 (1952). – W. A. D. ANDERSON, Pathology (Mosby Co., St. Louis 1953). – B. ENGFELDT and A. ENGSTRÖM, Acta orthoped. Scand. 24, 85 (1954). – A. ENGSTRÖM and L. WEGSTEDT, Acta Radiol. 35, 345 (1951). – J. D. KOUCKY, Ann. Surg. 81, 821 (1925).

Evidences of Cytological Basis of Differentiation

Recently, with the discovery of the endopolyploid nature of differentiated cells¹, attention has been directed towards chromosomal control of differentiation. But it is yet problematic how different degrees of polyploidy can account for qualitative differences between different organs.

Investigations on this problem, carried out on plants of different groups in this laboratory², have revealed the presence of chromosome numbers different from the normal $2n$ ones in differentiated organs. Of the numerous plants so far worked out, the results obtained from three species of *Menispermaceae* are discussed here. The particular differentiated organ studied in the present case was the leaf.

The $2n$ chromosome number, as studied from the root-tip cells, is twenty-six in *Tinospora cordifolia*. In the meristematic region of the stem-tips of this species, varying chromosome numbers have been observed, namely, twelve, thirteen, fourteen and twenty-six, the last number occurring with the highest frequency. The occurrence of such varying numbers in the meristematic portion seems to involve somatic reduction, as in a number of cases, clear thirteen and thirteen chromosomes (not chromatids) in anaphase could be seen. In the leaf, on the other hand, thirteen chromosomes are found to be the number present in all the cells. The thirteen chromosomes of the leaf do not represent the haploid complement, as is clear from a glance at their chromosome morphology. The lower numbers in the stem-tip do not represent the haploid set, implying thereby that the reduction in number occurs at random.

In *Stephania hernandifolia*, the numbers in the stem-tip vary from eighteen, twenty to twenty-two and the number present in the leaf-tip is twenty. In *Cocculus villosus*, similar variant chromosome numbers have been found in the stem-tip, such as, eighteen, twenty and twenty-two and the leaf-tip number is eighteen. The reported $2n$ numbers of both the species, as counted from root-tip cells, is twenty-two. For a study of the chromosomes of leaf and stem-tip, a special technique³ had been devised, which is awaiting publication.

In order to have a check of the chromosome number at the meristematic regions of the roots of all the three species, they were carefully reinvestigated. It was found that there too, although the reported numbers occur in the highest frequency in most of the cells, cells with other numbers are present, although with lower frequency.

The chromosomal control of differentiation is, therefore, clear from these investigations. It may be considered that the meristematic regions have the potentiality of differentiating into different organs. The differentiation is effected through the entrance of different chromosome numbers into specific organs of the plant body. There must be a screening or selective mechanism operating within the body, which is yet to be explored.

It may be postulated, therefore, that:

(1) Differentiation is controlled through chromosomes.

¹ L. GEITLER, Chromosoma 3, 271 (1948). – C. L. HUSKINS and L. M. STEINITZ, J. Hered. 39, 67 (1948). – E. THERMAN, Ann. Acad. Sci. Fenn. 4, 5 (1951).

² A. K. SHARMA and S. K. SARKAR, Sci. Cult. 22, September (1956).

³ A. K. SHARMA and ARCHANA SHARMA (nee MOOKERJEA), 1956 (in press).

(2) 'The different mature organs of the individuals, belonging to a species, contain different chromo-

Similar results are being obtained in a large number of plants and a detailed paper is being sent for publication

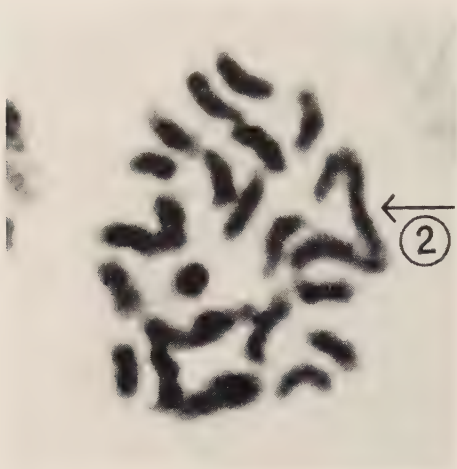


Fig. 1

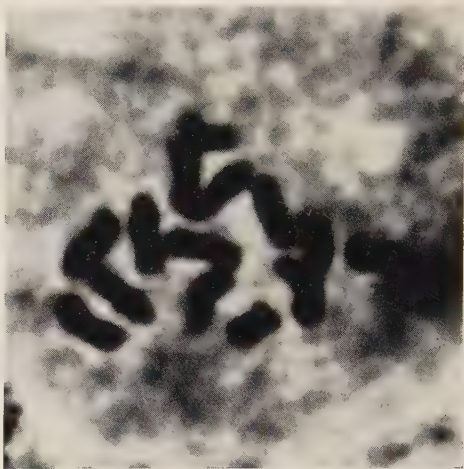


Fig. 4

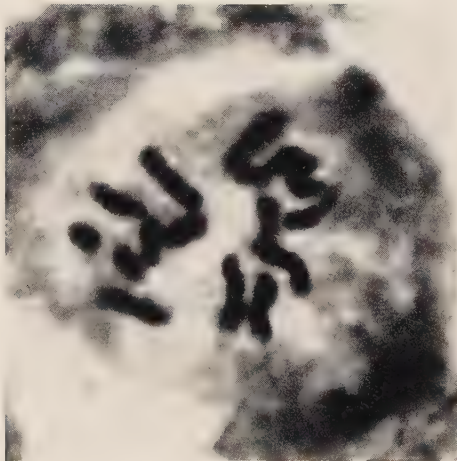


Fig. 2

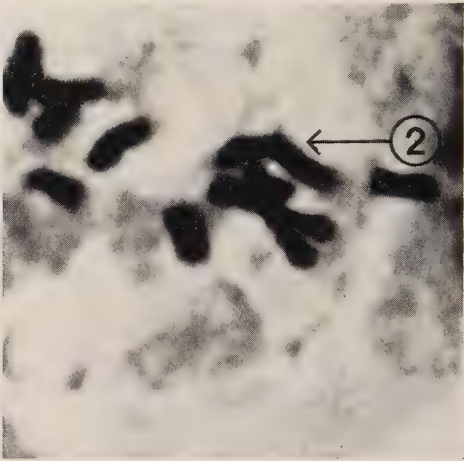


Fig. 5

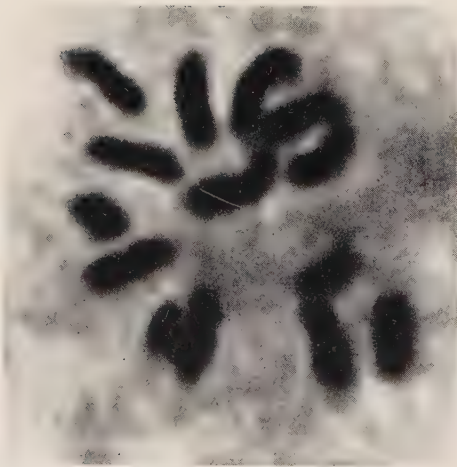


Fig. 3

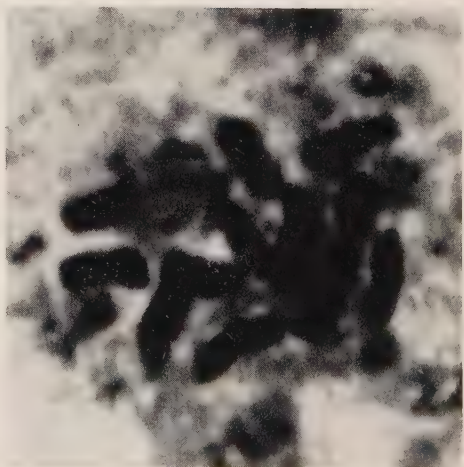


Fig. 6

somes complex specific to each organ, depending on the number and type of chromosomes responsible for their differentiation.'

As regards the origin of such varying numbers from the normal complement, somatic reduction seems to be principally responsible in the present case. In a previous

communication on palms² from this laboratory, where the differentiated organ studied was the basal portion of the

stem, evidences were gathered showing that 'partial endomitosis of chromosomes was responsible for differentiation of that particular organ'. A normal resting nucleus was found to lie beside the dividing smaller one with lesser chromosome number in the basal portion of the stem. This organ, as is well-known, can revert back to meristematic activity and give rise to lateral roots at times, obviously due to the regained activity of this normal nucleus. It is, therefore, clear that the means of origin of such varying numbers, whether through somatic reduction or partial endomitosis, is controlled by the fact of whether or not the organ concerned is to retain its potentiality of giving rise to normal meristematic nuclei at times of necessity. In the present case, as the organ concerned is the leaf, which is not endowed with the potentiality of giving rise to roots under any condition, the origin of such varying number is through somatic reduction.

ARUN KUMAR SHARMA and
ARCHANA SHARMA (Nee MOOKERJEA)

Cytogenetics Laboratory, Botany Department Calcutta University, Calcutta 19, December 11, 1956.

Zusammenfassung

Bei verschiedenen Pflanzenarten werden die Variationen der Chromosomenzahlen im Vegetationspunkt des Stammes untersucht. Für Blattzellen ist eine konstante Zahl charakteristisch. Die Resultate weisen darauf hin, dass in der Wachstumsspitze spezifische Chromosomenzahlen in die einzelnen Organe übergehen und so die Differenzierung gesteuert wird. Die normale $2n$ -Zahl kommt in den meisten Zellen vor.

Sur les caractéristiques histologiques des petits vaisseaux pulmonaires

Nous avons insisté récemment sur la nécessité d'améliorer par une technique appropriée les bases morphologiques de nos connaissances dans le domaine de la circulation du sang¹. Certains résultats histologiques acquis dans des conditions de préparations optimales, nous ont permis, en effet, de confronter immédiatement des détails structuraux de la paroi vasculaire mis en évidence sur une coupe fixée avec les observations cinématographiques de la circulation dans des organes transparents.

Il existe, cependant, une région circulatoire qui fait obstacle à une telle confrontation, puisque, d'une part, elle n'est pas accessible à l'examen cinématographique, et, d'autre part, les vaisseaux s'y trouvent sous l'influence de pressions, mécaniques extramurales les plus variables: c'est la circulation pulmonaire. Il faut se rendre compte que même en évitant les artefacts dûs aux manipulations préparatoires non convenables et à un prélèvement tardif du matériel, il reste ceux provoqués par la forme de l'agonie de l'organisme à l'instant même du prélèvement. Ce sont ces difficultés qui expliquent pourquoi, en dépit de tous les efforts poursuivis depuis près d'un siècle, l'histologie normale des petits vaisseaux pulmonaires est encore aujourd'hui une *terra incognita*.

Au cours des études s'attachant à certaines observations physiologiques de LECOMTE *et al.* sur le choc anaphylactique au niveau de la circulation pulmonaire², nous

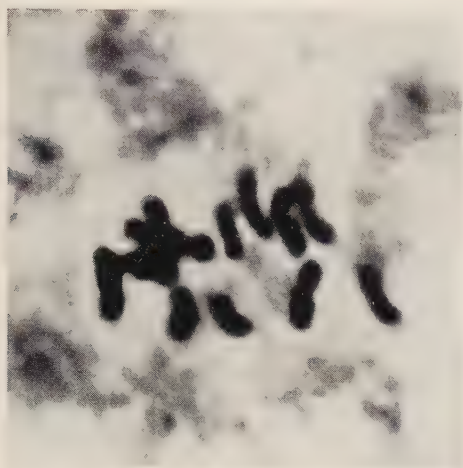


Fig. 7

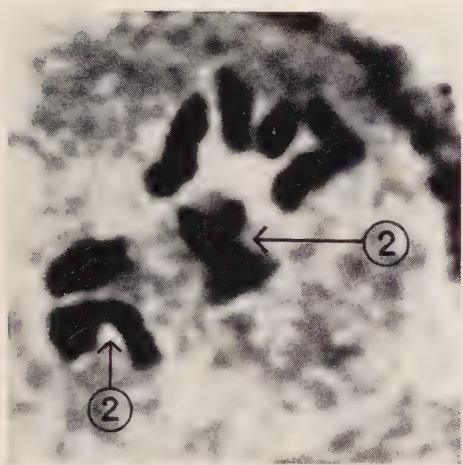


Fig. 8

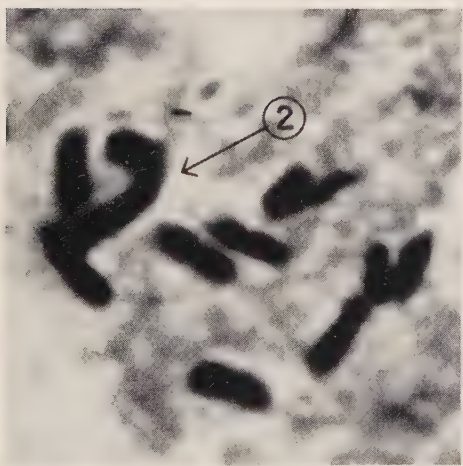


Fig. 9

Explanation of Figures: Stem-tip and Leaf-tip cells of *Tinospora cordifolia* showing varying number of chromosomes.

② ← indicates two overlapping chromosomes.

¹ S. HIRSCH, Exper. 11, 369 (1955); Acta med. Scand. 152, 379 (1955); Presse méd. 62, 978 (1954).

² J. LECOMTE, C. r. Soc. Biol. 150, 593 (1956). – J. LECOMTE et J. HUGHES, Arch. int. Allergy 8, 72 (1956).

avons appliqué notre technique histologique dans les conditions dites optimales. Grâce à ce procédé nous avons pu observer chez les lapins normaux des détails structuraux dans les petits vaisseaux pulmonaires qui n'ont pas été décrits dans la littérature.

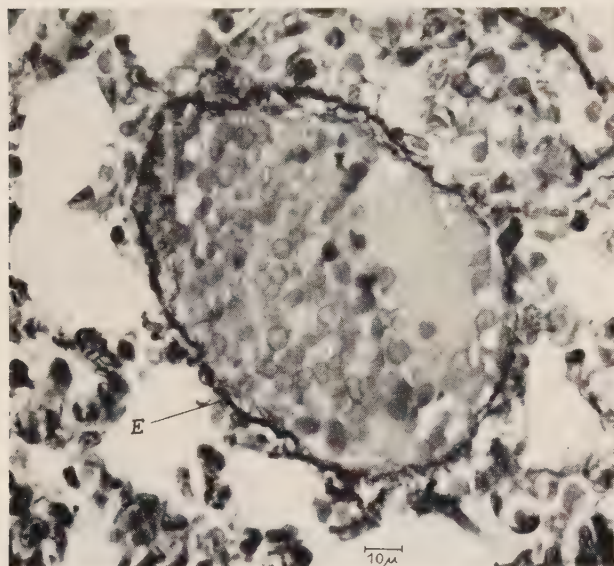


Fig. 1. Veinule pulmonaire pourvue d'une paroi élastique (E). Le remplissage de la lumière vasculaire indique qu'il s'agit d'une préparation dans des conditions optimales. Coupe de paraffine. Color.: résorcline-fuchsi-ne-hématoxyl.

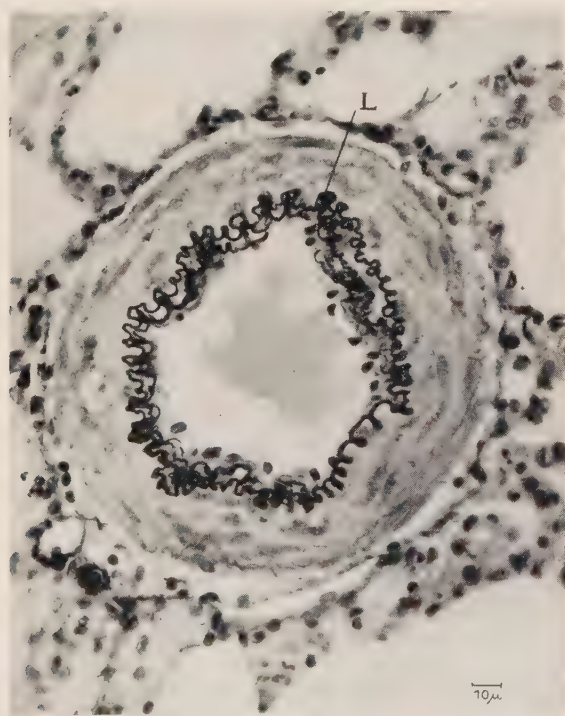


Fig. 2. Petite artère pulmonaire du lapin. Multiplication et dédoublement des lamelles élastiques (L). Technique voir Fig. 1.

Nous avons pu établir que les veinules pulmonaires du lapin et également du cobaye, à l'encontre des veinules

de la circulation générale, possèdent une paroi composée de tissu élastique (Fig. 1).

Ensuite, nous avons constaté sur les images des petites artères pulmonaires des lapins en état normal, des dédoublements et des multiplications des lamelles

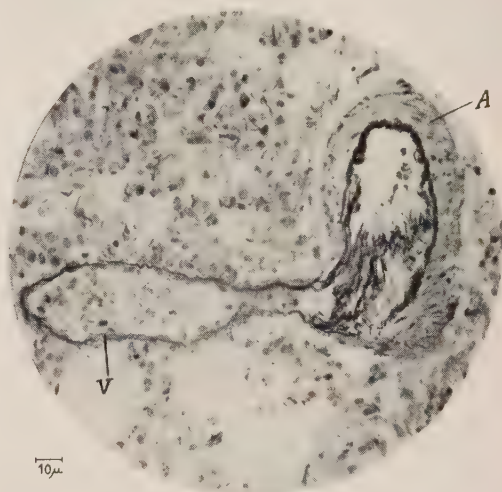


Fig. 3. Anastomose artério-veineuse dans le poumon du lapin. Secteur artériel (A) en état de contraction partielle. Secteur veineux: V. Technique voir Fig. 1.

élastiques rappelant les modifications décrites comme élastose proliférative dans la littérature histopathologique de l'homme (Fig. 2).

Enfin, nous avons pu mettre en évidence les rapports des petites branches artérielles pulmonaires appelées généralement «Sperrarterien» avec le système veineux par des anastomoses artério-veineuses. Ces rapports sont connus depuis les observations de v. HAYEK³. Cependant, l'aspect sous lequel nous avons pu les vérifier (Fig. 3) semble confirmer la thèse exposée dans nos communications précédentes, c'est-à-dire, que les «Sperrarterien» ne constituent pas un type artériel particulier pourvu de dispositifs de fermeture, mais bien, en réalité, des artères dont la musculature est fixée, à l'instant même de leur contraction.

Le fait que les parois des veinules pulmonaires de certains rongeurs sont formées de tissu élastique, s'il se confirmait pour l'homme – et nous avons des raisons de le croire – peut ouvrir une perspective intéressante non seulement au point de vue morphologique, mais aussi pour les concepts ayant trait à la physiologie et à la pathologie de la respiration. En ce qui concerne les dédoublements et les multiplications des lamelles élastiques nous gardons une certaine réserve quant à leur interprétation précise. Toutefois leur observation suggère une révision des interprétations courantes sur la soi-disant élastose proliférative des petites artères, notamment au niveau de la circulation rénale et attire l'attention sur des facteurs d'ordre *fonctionnel* qui pourraient être à l'origine de telles images.

S. HIRSCH

Laboratoire d'Anatomie pathologique de la Faculté de Médecine de l'Université de Bruxelles, le 15 décembre 1956.

³ H. v. HAYEK, Z. Anat. 110, 412 (1940).

Zusammenfassung

Histologische Untersuchungen, die unter lebensnahen Bedingungen am Kaninchen durchgeführt wurden, zeigen, dass die Wand der kleinsten Lungenvenen aus elastischem Gewebe besteht. In der Wand der kleinen Lungenarterien bestehen unter normalen Verhältnissen Verdoppelungen und Vervielfachung der elastischen Lamellen, die an die als proliferative Elastose in der menschlichen Pathologie beschriebenen Veränderungen erinnern. Bilder von in Funktion befindlichen arteriovenösen Anastomosen deuten darauf hin, dass die sogenannten Sperrarterien der Lunge keine mit «Sondervorrichtungen» versehenen Gefässe sind, sondern dass es sich um Arterien handelt, deren Muskulatur im Kontraktionszustand fixiert ist.

Homologe Interferenz durch hitzeinaktiviertes, an Erythrozyten adsorbiertes Influenza-B-Virus

Die Beobachtung von FRANCIS¹, dass auch inaktiviertes Influenzavirus in der Chorioallantois des Hühnerembryos das Phänomen der sogenannten Interferenz auszulösen vermag, wurde oft bestätigt. Die meisten Autoren sind der Ansicht, dass die Interferenz darauf beruhe, dass das vorher in grosser Dosis applizierte inaktive, das heisst nicht vermehrungsfähige Virus, die empfänglichen Zellen gegen das nachher eingeführte lebende Virus schütze, sei es durch die Besetzung der an der Zelloberfläche gelegenen Rezeptoren, sei es durch die Belegung eines intrazellulären Faktors, der für die Vermehrung der aktiven Virus notwendig ist. PRICE *et al.*², die das Interferenzphänomen bei Rickettsien nachwiesen, kamen zum Schluss, dass es sich dabei nicht um den erfolgreichen Wettlauf der inaktivierten Rickettsien um die empfänglichen Zellen handle. Nach diesen Autoren spielt die Anzahl der empfänglichen Zellen für das Zustandekommen der Interferenz keine Rolle, sondern allein das Verhältnis zwischen der Anzahl der inaktivierten zu den lebenden Rickettsien, die den Versuchstieren appliziert werden. Sowohl lebendes als inaktiviertes Influenzavirus wird auch an die Oberfläche von Erythrozyten adsorbiert, wobei dieselben agglutiniert werden. Während ersteres nach der Zerstörung der Rezeptorsubstanz der Erythrozyten frei wird, um gegen frische Erythrozyten wieder volle Aktivität zu zeigen (HIRST³), löst sich inaktiviertes Virus nicht wieder ab (BURNET⁴; BRIODY⁵). Wenn die Hypothese richtig ist, dass die Interferenz von der Besetzung der Allantoisendothelien abhängt, dann sollte an Erythrozyten gebundenes inaktiviertes Virus nicht instande sein, die Entwicklung des später applizierten lebenden Virus zu hemmen. Im folgenden wird gezeigt, dass sich diese Annahme nicht bestätigte, dass vielmehr das Einbringen von an Erythrozyten adsorbiertem inaktiviertem Virus in die Allantoishöhle die Entwicklung des später applizierten lebenden Virus zu hemmen vermag. Von den zahlreichen Experimenten, die ausgeführt wurden, sei eines ausführlich wiedergegeben.

Experimentelles. Die Versuche wurden mit einem Stamme von Influenza B ausgeführt, der im März 1955

während einer Epidemie in Zürich isoliert worden war. Die Inaktivierung des Virus geschah nach der Methode von ISAACS und EDNEY⁶: 6 Teile virushaltiger Allantoisflüssigkeit, 2 Teile zweiprozentiges Natriumzitrat in physiologischer NaCl-Lösung, 1 Teil Natriummetaboratpuffer pH 8,5; die Mischung während einer Stunde im Wasserbad bei 56°C inaktivieren. Der Agglutinationstiter gegenüber einer 0,5%igen Aufschwemmung von Hühnererythrozyten vor Inaktivierung war 2048, nach Inaktivieren 1000.

20 cm³ des inaktivierten Virus wurden mit 0,2 cm³ gründlich gewaschener Hühnererythrozyten versetzt und gut gemischt. Es trat sofort komplette Agglutination auf. Nach halbstündiger Einwirkung und mehrmaligem Aufschütteln wurden die Blutkörperchen ausgeschleudert. Titer der Allantoisflüssigkeit nach Absorption: 32. Die Blutkörperchen wurden zweimal mit 10 cm³ NaCl-Lösung gewaschen und darauf in 10 cm³-NaCl-Lösung aufgenommen. In die Allantoishöhle von 6 während 10 Tagen bebrüteten Eiern wurde je 0,5 cm³ der mit dem inaktivierten Virus beladenen Erythrozyten injiziert, 6 Eier erhielten die gleiche Dosis normaler Erythrozyten und 6 Eier 0,5 cm³ NaCl-Lösung. Nach 24 h im Brutschrank wurde in die Allantoishöhle aller Eier 0,1 cm³ aktives Virus 1:1000 verdünnt inokuliert. Nach weiteren 48 h bei 35°C wurden die Eier über Nacht bei etwa 4°C gehalten und dann die Allantoisflüssigkeit entnommen. Sie ergaben folgende Agglutinationstiter:

Mit beladenen Erythrozyten behandelte Eier	Mit normalen Erythrozyten behandelte Eier	Mit NaCl behandelte Eier
8	512	1024
0	1024	2048
4	1024	1024
8	512	512
0	2048	2048
16	1024	1024

In einigen der Experimente, die in der Anordnung obigem Beispiel entsprachen, zeigte gelegentlich eine der Allantoisflüssigkeiten der mit beladenen Erythrozyten vorbehandelten Eier einen Agglutinationstiter von 128 bis 1:256 bei 4–8fach höheren Titern der Kontrollen. Der Grad der Hemmung war abhängig von der Dauer des Brutschrankaufenthaltes zwischen der Einspritzung der mit inaktiviertem Virus beladenen Erythrozyten und der Inokulation des aktiven Virus. Wenn das aktive Virus nur zwei Stunden nach den beladenen Erythrozyten inokuliert wurde, zeigten die Allantoisflüssigkeiten meistens nur partielle Hemmung gegenüber den Kontrollen. Diese Latenzzeit bis zum vollen Wirksamwerden des an Erythrozyten adsorbierten inaktivierten Virus lässt darauf schliessen, dass in der Allantoishöhle etwas von den beladenen Erythrozyten abgegeben wird, das für die Interferenz verantwortlich ist. Bei aufrecht inkubierten Eiern fanden sich die Erythrozyten im Grunde des Allantoissackes gesammelt. Sie waren offenbar nur mit relativ wenigen Allantoiszellen in Berührung gekommen.

H. MOOSER und J. LINDENMANN

Hygiene-Institut der Universität Zürich, den 3. Januar 1957.

⁶ A. ISAACS und M. EDNEY, Austr. J. exp. biol. med. Sci. 28, 219 (1950).

¹ T. FRANCIS, jr., J. exp. Med. 85, 1 (1947).

² W. H. PRICE, J. W. JOHNSON, H. EMERSON und C. E. PRESTON, Science 120, 457 (1954).

³ G. K. HIRST, J. exp. Med. 76, 195 (1942).

⁴ F. M. BURNET, Austr. J. exp. biol. med. Sci. 30, 119 (1952).

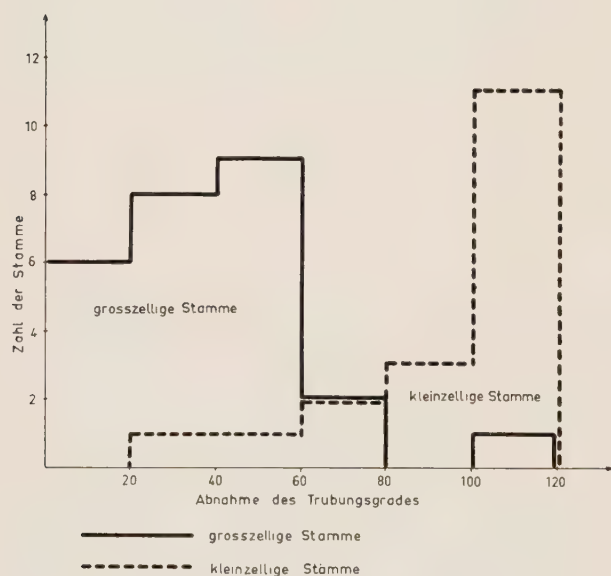
⁵ B. A. BRIODY, J. Immunol. 59, 115 (1948).

Summary

Influenza B Virus, inactivated according to the method of ISAACS and EDNEY and adsorbed onto chicken red cells, interfered with the growth of active virus in the chorioallantois of the chick embryo.

Lysozymempfindlichkeit der Spezies *B. megaterium*

Die elektive Auflösung der Zellwand und die Freisetzung der Protoplasten zufolge Lysozymeinwirkung wurde von TOMCSIK und GUÉX-HOLZER¹ sowie von WEIBULL² an zwei Stämmen von *B. megaterium* (Bazillus M bzw. KM) nachgewiesen. Das Verhalten der Spezies *B. megaterium* gegenüber Lysozym ist hingegen noch unbekannt. In der vorliegenden Arbeit wird über die Lysozymempfindlichkeit von 54 *B. megaterium*-Stämmen berichtet, wovon 46 von uns aus dem Boden isoliert wurden (TOMCSIK und BAUMANN-GRACE³).



Lysozymverdauung der gross- und kleinzelligen *B. megaterium*-Stämme.

Zum Studium des lytischen Effektes des Lysozyms wurden die bei 28°C während 16 h auf Agar gewachsenen Bakterien in physiologischer NaCl-Lösung derartig suspendiert, dass die Suspensionsdichte im Klett-Summersson photoelektrischen Kolorimeter, mit Blaufilter Nr. 42 gemessen, 180 betrug. Zu 3 ml dieser Suspension wurde 1 ml 1:2000 kristallisiertes Lysozym (Armour) gegeben und das Gemisch bei 37°C 1 h bebrütet. Die Kontrollröhrchen ohne Lysozym zeigten bei 13 Stämmen eine Autolyse verschiedenen Grades; deshalb konnte die Lysozymempfindlichkeit dieser Stämme nicht ausgewertet werden. Die Resultate mit den anderen Stämmen wurden in der Abbildung graphisch zusammengefasst.

¹ J. TOMCSIK und S. GUÉX-HOLZER, Schweiz. Z. Path. Bakt. 15, 517 (1952).

² C. WEIBULL, J. Bact. 66, 688 (1953).

³ J. TOMCSIK und J. B. BAUMANN-GRACE, Verh. Naturf. Ges. Basel 67, 218 (1956).

Wie aus der Abbildung klar ersichtlich, zerfallen die untersuchten *B. megaterium*-Stämme, in ihrem Verhalten gegenüber Lysozym, in zwei mehr oder weniger scharf getrennte Untergruppen. Die phasenkontrastmikroskopische Kontrolle ergab, dass die überwiegende Mehrzahl der schwache Lyse aufweisenden Stämme grosszellig war, mit einem Zellendurchmesser von 1,2 bis 1,5 μ . Die Zellen enthielten reichlich Fetttropfchen in Form von Inklusionskörperchen, und ihre Zellwand wurde durch Lysozym nur teilweise verdaut. Demgegenüber war die überwiegende Mehrzahl der Stämme mit einem Zellendurchmesser von 0,9 bis 1,2 μ in hohem Grade lysozymempfindlich. Diese kleinzellige Untergruppe enthielt keine oder nur spärlich Fettinklusionskörperchen, und ihre Zellwand wurde vollkommen aufgelöst. Die früher eingehend studierten «Bacillus-M»- und KM-Stämme gehörten in die kleinzellige Untergruppe.

Das Verhalten gegenüber Lysozym wurde weiterhin bei 53 Stämmen auch in 10,2% Saccharoselösung nach WEIBULL² untersucht. Die Endkonzentration des Lysozyms betrug bei kristallisiertem Lysozym 1:32 000, bei Eiklar 1:800. Die bei Zimmertemperatur belassenen Gemische wurden während 1½ h alle 15 min im Phasenkontrast untersucht. In 20–30 min wurden bei 28 Stämmen nach vollkommener Auflösung der Zellwand zellwandfreie Protoplasten freigesetzt, die bei 4°C mindestens 24 h eine Stabilität aufwiesen. Alle diese Stämme gehörten zur kleinzelligen Untergruppe. Bei den anderen Stämmen konnte wohl das Anfangsstadium der Protoplastenabrundung mit Abhebung der Zellwand beobachtet werden, doch erfolgte die Auflösung der Zellwand nur in kleinem Masse. Die an und für sich häufig unsichtbaren Zellwände wurden mit Hilfe der spezifischen Zellwandreaktion (TOMCSIK und GUÉX-HOLZER⁴) deutlich dargestellt. Die nur zum Teil freigesetzten Protoplasten waren zumeist von grosser Gestalt und zeigten geringe Stabilität. Durch Erhöhung der Lysozymkonzentration und nach Verlängerung der Inkubationszeit konnte die Gewinnung zellwandfreier Protoplasten in dieser meistens grosszelligen Untergruppe kaum gefördert werden.

Für diese Arbeit wurde ein Beitrag aus Arbeitsbeschaffungskrediten des Bundes zur Verfügung gestellt.

JOYCE B. BAUMANN-GRACE und
J. TOMCSIK

Hygienisches Institut der Universität Basel, den 28. Januar 1957.

Summary

The 54 strains of *B. megaterium* examined could be divided broadly into 2 groups on the basis of their lysozyme sensitivity. In most of the large-celled strains the cell walls were incompletely digested. Loss of turbidity on addition of lysozyme was comparatively slight and few free protoplasts were formed in the presence of saccharose. In contrast, the small-celled strains usually showed a marked drop in turbidity and complete protoplast formation could be obtained.

These results suggest that, in some strains of *B. megaterium*, particularly those possessing very large cells, certain substances are included in the cell walls which are not depolymerized by lysozyme.

⁴ J. TOMCSIK und S. GUÉX-HOLZER, J. gen. Microbiol. 10, 317 (1954).

Über den Vitaminhaushalt von Kolkulturen in vitaminfreien und vitaminhaltigen Nährlösungen

1. *Einleitung.* Es ist bekannt, dass Kolkulturen zur Synthese einer Reihe von B-Vitaminen befähigt sind¹. Andererseits wurde nachgewiesen, dass *Escherichia coli* Vitamin B₁₂ aus seiner Umgebung adsorbieren kann². Wir prüften die Frage, ob einem flüssigen Nährboden auch andere B-Vitamine entzogen werden, wenn der Nährboden diese Vitamine in physiologischen Mengen bereits enthält, oder ob der Nährboden aus der bakteriellen Synthese weiter angereichert wird. Zur Kontrolle liefen Untersuchungen an vitaminfreien Nährlösungen parallel.

2. *Methodik.* Zu den Untersuchungen wurden Kolkulturen in vitaminisiertem und nichtvitaminisiertem Nährboden 18 h bei 37°C bebrütet. Zur Technik sei auf ¹ verwiesen. Nach 18 h befinden sich die Kulturen in der logarithmischen Vermehrungsphase, dem Zustand der stärksten Vermehrung. Dann wurde die Kultivierung abgebrochen. Ein Aliquot der Kulturflüssigkeit wurde nach Sterilfiltration ohne weitere Vorbehandlung, ein Anteil der bakterienhaltigen Gesamtkultur nach der für die einzelnen Vitamine spezifischen Extraktion mikrobiologisch auf den Gehalt an einigen B-Vitaminen untersucht. Die von uns verwendeten Vitaminextraktionen siehe unter H. HAENEL³. Auf diese spezifischen Extraktionsmethoden konnte nicht verzichtet werden; einständige Behandlung im strömenden Dampf zum Beispiel setzte nur einen Teil der nach Extraktion erfassbaren Vitaminmengen frei.

3. *Ergebnisse.* Einige repräsentative Resultate sind in der folgenden Tabelle zusammengestellt.

4. *Diskussion.* Danach war die Abnahme der im Nährboden gelöst enthaltenen Vitamine unter dem Einfluss der Bakterienzellen unterschiedlich. Sie betrug für Biotin, Nikotinsäure, Vitamin B₆ und Vitamin B₁₂ zwischen 5 und 87% und für Thiamin 46%. Pantothensäure und Inosit nahmen um 11 bzw. 16% ab. Folsäure wurde nicht vermindert.

Inosit wird von *E. coli* im Energiestoffwechsel nicht verlegt, dürfte auch keine Bedeutung als Wirkstoff besitzen. In Inosit-freien Nährlösungen wurde synthetisiertes Inosit weder im Filtrat noch in der bakterienhaltigen Kultur gefunden (Tabelle). Trotzdem scheint ein Teil des Inosits von den Zellen adsorbiert worden zu sein. Das würde bedeuten, dass im Darm auch solche Wirkstoffe dem Wirt entzogen werden können, die im Bakterienstoffwechsel entbehrlich sind.

Kolibakterien, wie vermutlich auch andere Bakterien, sind demnach zwar zur Eigensynthese einer Reihe von B-Vitaminen befähigt und können aus dieser Synthese gewisse Mengen an den Nährboden abgeben. In vitaminhaltigen Nährlösungen dagegen kann diese Abgabe unterbleiben und es kann im Gegenteil ein Verbrauch der im Nährboden vorgelegten Vitamine stattfinden.

Das würde, auf die Verhältnisse im Darmkanal übertragen, bedeuten, dass bestimmte Bakterien ihren Vita-

Vitamingehalt von Kolkulturen, die in vitaminhaltigen (a) und vitaminfreien (b) synthetischen Nährlösungen 18 h bei 37°C gezüchtet wurden, sowie der Einfluss der Kultivierung auf den Vitamingehalt der zellfreien Kulturfiltrate.

	Vitamin- gehalt des unbeimpf- ten Nähr- bodens	Vitamin- gehalt des Kulturfil- trates nach 18 h Bebrü- tung b. 37°C	Vitamin- verlust im Filtrat nach 18 h Bebrü- tung bei 37°C %	Vitamin- gehalt der Gesamt- kultur nach spezif. Ex- traktion der Vitamine
Biotin { a b	0,2 0,008	0,032 0,048	84	1,22 0,8
Nikotinsäure { a b	168 3	22,5 12	87	193 48,2
Folsäure { a b	4,1 0,25	4 1,15	0	17 20
Vitamin B ₆ { a b	45 3,3	8,4 11	81	57,6 15,9
Inosit { a b	38,8 0	32,4 0	16	45,6 0
Thiamin { a b	210 0	114 0	46	232 31,5
Vitamin B ₁₂ { a b	0,04 0,003	0,0092 0,008	77	0,061 0,028
Pantothensäure { a b	44 2,5	39,2 77,2	11	64,5 69

Angaben in ng (10⁻⁹ g), bei Inosit in µg (10⁻⁶ g), je ml Nährboden bzw. Kultur.

minbedarf aus der Eigensynthese, aber auch aus exogenen Quellen decken können.

H. HAENEL und WALHEIDE MÜLLER-BEUTHOW

Anstalt für Vitaminforschung und Vitaminprüfung,
Potsdam-Rehbrücke, den 27. November 1956.

Summary

A strain of *E. coli*, when cultivated in a vitamin-free medium, delivered from its synthesis small amounts of biotin, nicotinic acid, folic acid, vitamin B₆, pantothenic acid and occasionally vitamin B₁₂ into the cell-free filtrate, but not thiamine or inositol. Cultivated in a vitamin-containing medium the *E. coli* strain absorbed certain amounts of biotin, nicotinic acid, vitamin B₆, thiamine and vitamin B₁₂, while inositol, folic acid and pantothenic acid were absorbed only in small amounts or not at all.

Species Difference in Trapped Plasma of the Packed Red Cell Column

Although a species difference has been demonstrated in the packed red cell column¹ (PCV) it seems that insufficient attention is paid to this when investigating

¹ F. W. JENNINGS, I. M. LAUDER, W. MULLIGAN, and G. M. URQUHART, Vet. Rec. 66, 155 (1954).

¹ H. HAENEL und W. MÜLLER-BEUTHOW, Klin. Wschr. 34, 643 (1956).

² P. BURKHOLDER, Science 114, 459, 478 (1951). – E. HOFF-JØRGENSEN, A. P. SKONBY und J. G. ANDRESEN, Nord. Med. 48, 754 (1952). – J. JÄNNES, Acta physiol. Scand. 31, 183 (1954).

³ H. HAENEL, Pharmazie 9, 489 (1954).

characteristics of erythrocytes of different animals. GOODWIN² using the PCV in the calculation of intracellular glucose has drawn important conclusions which might have been different if observed haematocrit values had been corrected for plasma trapping in accordance with the species of blood used. Similarly, failure to take account of the typical amount of plasma trapping in estimations of ionic concentrations, glycolytic rate and other characteristics of erythrocytes, when calculations involve the use of observed PCV, could lead to erroneous results. This is especially true for goat blood which was found to contain a considerable amount of plasma in the packed red cell column.

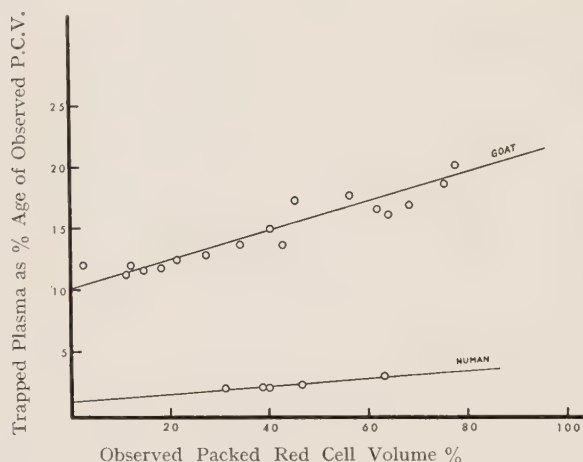


Fig. 1.—The amount of plasma in the packed red cell column of goat and of human blood expressed as a percentage of the observed packed red cell column.

Plasma trapping was estimated as described by CHAPLIN and MOLLISON³ with slight modifications. Preliminary investigation showed that goat erythrocytes were not affected by the high concentrations of EVAN'S Blue dye used⁴. Plasma trapping in the packed red cell column of fresh heparinized goat and human blood centrifuged in Wintrobe tubes at 1500 $\times$ G for 55 min varied directly with the height of the red cell column (Fig. 1).

The regression equation in the case of human blood was:

$$Y = 0.352 X + 0.839,$$

and in the case of goat blood:

$$Y = 0.119 X + 10.22,$$

where Y is the percentage plasma trapping and X the observed PCV as a percentage of whole blood. At an haematocrit level of 45% trapping for human blood was 2.4%, but for goat blood it was 15.6%.

It was possible to increase the degree of packing of red cells by increasing the time and force of centrifugation. This is demonstrated in Figure 2 which shows percentage decrease in observed PCV plotted against total force or 'impulse' measured in dyne-seconds. Actual determinations showed that this decrease was the result of diminishing amounts of trapped plasma and not of loss of cell contents. However, it would be impracticable to

increase centrifugation to bring about the same degree of packing of goat cells as of human cells centrifuged at 1500 $\times$ G for 55 min. It is therefore important when

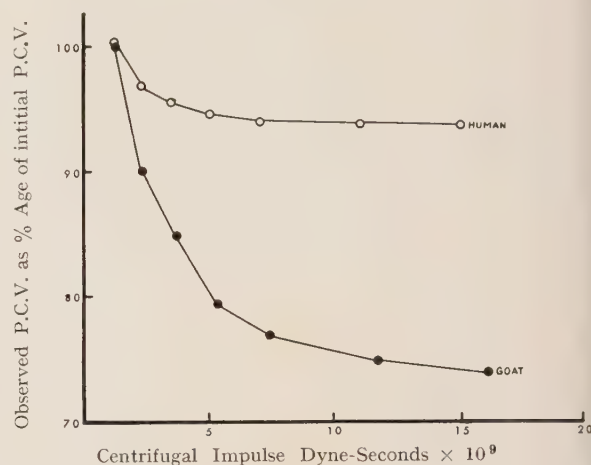


Fig. 2.—The effect of varying centrifugal force on the observed packed red cell volume of human and of goat blood.

centrifuging under usual laboratory conditions to make an accurate allowance for plasma trapping according to the species of blood under investigation.

G. R. WADSWORTH

Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London, December 17, 1956.

Zusammenfassung

Nach Zentrifugieren unter gleichen Bedingungen enthält bei Ziegenblut die Erythrozytensäule ungefähr siebenmal soviel eingeschlossenes Plasma wie bei Menschenblut. Mit Hilfe der angegebenen Formeln können diese Plasmamengen berechnet werden; dabei werden die Resultate in Hämatokritwerten ausgedrückt. Es ist wichtig, bei der Berechnung der Eigenschaften von Erythrozyten diese Unterschiede zwischen den verschiedenen Spezies in der Menge des zurückgehaltenen Plasmas zu berücksichtigen.

The Biosynthesis of 6-Azauracil Riboside by *Escherichia coli* Growing in the Presence of 6-Azauracil

Recently we have found that 6-azauracil (3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine) inhibits the growth of a number of microorganisms (ŠORM and ŠKODA¹; cf. also HANDSCHUMACHER and WELCH²). We further found that the inhibitory effect on the growth of *E. coli* can be completely removed by uracil, cytosine, or uridine, and that the inhibition is strictly competitive in character³. We

¹ F. ŠORM and J. ŠKODA, Chem. listy 50, 827 (1956); Coll. Czechoslov. Chem. Commun. 21, 487 (1956).

² R. E. HANDSCHUMACHER and A. D. WELCH, Federation Proc. 15, Abstract No. 871 (1956).

³ J. ŠKODA and F. ŠORM, Chem. listy 50, 1165 (1956); Coll. Czechoslov. Chem. Commun. 21, 1328 (1956).

² R. F. W. GOODWIN, J. Physiol. 134, 88 (1956).

³ H. CHAPLIN and P. L. MOLLISON, Blood 7, 1227 (1952).

⁴ G. R. WADSWORTH, Exper. 11, 394 (1955).

⁵ C. J. HLAD and J. H. HOLMES, J. appl. Physiol. 5, 457 (1953).

Dependence of 6-azauracil riboside production and culture growth on the concentration of 6-azauracil

Molarity of 6-azauracil	Growth (as percentage of maximum growth)	6-Azauracil riboside (as percentage of maximum production)
0	100	0
2×10^{-4}	58	29
4×10^{-4}	50	56
6×10^{-4}	44	85
8×10^{-4}	39	88
10^{-3}	37	100

have further been able to show that 6-azauracil has a cancerostatic effect on a number of experimental tumours (ŠORM, JAKUBOVIČ, and ŠLECHTA⁴; cf. also HAKALA, LAW, and WELCH⁵, ŠABLÍK and ŠORM⁶).

Some 'unnatural' purine and pyrimidine bases are known to be incorporated into nucleic acids by various biological systems (MATTHEWS⁷, ZAMENHOF and GRIBOFF⁸) evidently by way of the corresponding ribosides or desoxyribosides. Nucleosides of such 'unnatural' analogues have hitherto been isolated from nucleic acids only in the case of 5-bromouracil⁹; from 6-azathymine, a desoxyriboside was obtained by a transdesoxyribosidation reaction¹⁰. HANDSCHUMACHER and WELCH further found² that resting cells of *Streptococcus faecalis* incubated with 6-azauracil and thymidine accumulated a compound which had the properties expected of 6-azauracil desoxyriboside.

We have now found that cultures of *E. coli* B growing on media containing glucose and inorganic salts only, with the addition of sub-bacteriostatic concentrations of 6-azauracil, accumulate 6-azauracil riboside in the medium. The gradual disappearance of 6-azauracil from the medium, and the accumulation of its riboside, were followed by paper chromatography. The amount of the riboside formed at various concentrations of 6-azauracil, and the corresponding intensities of growth of the cultures, are recorded in the table.

With a view to isolating the 6-azauracil riboside, we carried out the stationary cultivation of *E. coli* in 10 l of medium containing a 8×10^{-4} M concentration of 6-azauracil, from a large inoculum. After removal of the cells, the azauracil riboside together with unchanged azauracil was adsorbed on active charcoal, eluted with 50% ethanol containing 1% ammonia and the residue obtained on evaporation of the eluate chromatographed on a column of cellulose powder with *n*-butanol saturated with water as the eluent. By this procedure, a sharp separation of 6-azauracil from its riboside was achieved. The 6-azauracil riboside was purified by a second chromatography in the same system and analyzed by Analysis, Calculated for $C_8H_{11}N_3O_6$: C 39.18%; H 4.52%; N 17.13%; Found: C 38.81%; H 4.82%; N 16.84%.

Phosphorus was absent. In paper electrophoresis, the isolated product behaved as a homogeneous substance resembling uridine in its electrophoretic mobility. The



Chromatogram of a hydrolysate of 6-azauracil riboside (photographed by UV-light). Whatman No. 1 paper, developed with *n*-butanol saturated with water. 1 uracil, 2 6-azauracil, 3 hydrolysate of 6-azauracil riboside (4 N-HCl, 15 h, 105°C), 4 6-azauracil riboside, 5 uridine.

ultraviolet spectrum of 6-azauracil riboside shows $\lambda_{\max}$ 262 m μ , ϵ 6.1×10^{-3} (in water, while 6-azauracil has $\lambda_{\max}$ 258 m μ , ϵ 5.4×10^{-3} , in water). After hydrolysis with 4 N-HCl and chromatography a single base, identical with 6-azauracil, was detected (figure); chromatographic analysis after hydrolysis with $N-H_4SO_4$ revealed ribose as the sole sugar component.

Note added in proof: 6-azauracil riboside has now been obtained crystalline, m.p. 160–161°C.

This work and further experiments will be described in detail in the Collection of Czechoslovak Chemical Communications.

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Department of Biochemistry, Institute of Chemistry, Czechoslovak Academy of Science, Prague, December 13, 1956.

Zusammenfassung

Bei der Kultivierung von *E. coli* in 6-azauracilhaltigem Medium wird ein neuer Stoff akkumuliert, der als 6-Azauracilribosid identifiziert wurde.

⁴ F. ŠORM, A. JAKUBOVIČ, and L. ŠLECHTA, Exper. 12, 271 (1956).

⁵ M. T. HAKALA, L. W. LAW, and A. D. WELCH, Proc. Amer. Ass. Cancer Res. 2, 113 (1956).

⁶ J. ŠABLÍK and F. ŠORM, Neoplasma (in press).

⁷ R. E. F. MATTHEWS, Proceedings of the Third International Congress of Biochemistry, Bruxelles 1955 (Acad. Press, New York 1956), p. 63.

⁸ S. ZAMENHOF and G. GRIBOFF, Nature 174, 306 (1954).

⁹ F. WEYGAND, A. WACKER, and K. M. PATIL, Ber. 89, 475 (1956).

¹⁰ W. H. PRUSOFF, J. biol. Chem. 215, 809 (1955).

Distribution of Protease Activity in the Blastula and Early Gastrula of *Discoglossus pictus*

Recent investigations¹ have demonstrated in the early gastrula of *Amblystoma* a gradient of dipeptidase activity with a maximum in the blastoporal region. On the other hand, studies with labelled amino acids² have shown a higher rate of incorporation in the blastoporal lip than in any other region. These facts suggest that this region must be the site of a higher turnover of proteins. Therefore it seemed interesting to investigate the distribution of protease activity in the different territories of the blastula and early gastrula.

It should be mentioned here that determinations of protease activity on whole embryos³ have shown a decreasing rate of activity from fertilization to the neurula stage.

Females and males of *Discoglossus pictus* were injected with 400 I.U. (400 I.U./1 mg) of Gonadotrop Schwang kindly supplied by the Firma CIBA.

Fertilized eggs were usually obtained 20 h after the injection and were kept at room temperature until they reached the desired stage.

Blastulae at stage 9 of SHUMWAY⁴, i.e. late blastulae, and gastrulae at stage 10, i.e. accumulation of pigment at the site of the blastoporal lip, were used. The embryos were dissected in concentrated HOLTFRETER'S solution, according to Figures 1 and 2. Blastulae were dissected in half, along the equator, thus obtaining an animal and a vegetal half. Early gastrulae were dissected as in Figure 2a, the two lateral regions being discarded. The yolk mass of the central sector was discarded with two cuts, as indicated in Figure 2b, and the dorsal part was then divided into two regions: region 1 which comprises presumptive chorda-mesoderm and presumptive neuroectoderm; and region 2, comprising only presumptive epidermis.

For each experiment, 30 embryos were dissected. The explants were rinsed in cold 0.65% NaCl solution in phosphate buffer 0.01 M, pH 7.4, and finally homogenized in 0.4 cm³ of the same solution.

Protease activity has been measured on the whole homogenate, according to DUSPIVA⁵. Preliminary experiments showed that the optimum for the protease activity of this material is at pH 4.9; hence all our assays were run at this pH.

Activity has been expressed as micromoles of Tyrosin released *per* 1 mg of N (total or cytoplasmic, as will be discussed presently), after 2 h of incubation at 30°C.

The problem of the reference in the calculation of enzymatic activity is a serious one in the case of Amphibian embryos. The unequal distribution of yolk between animal and vegetal halves, is likely to give rise to misleading results, should the enzymatic activity be calculated on the basis of total N. This question has been thoroughly discussed by GREGG and LØVTRUP¹, who have suggested to use as a reference the 'cytoplasmic Nitrogen', i.e. the Nitrogen which is extractable in the

above-mentioned NaCl solution, which in fact appears to leave unbroken both yolk platelets and pigment granules which can be removed by low-speed centrifugation (1700 × g for 15 min). In the present experiments, both

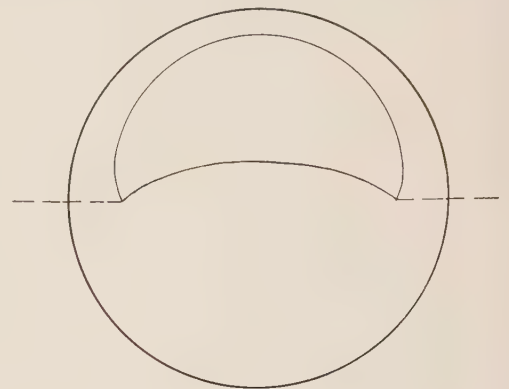


Figure 1

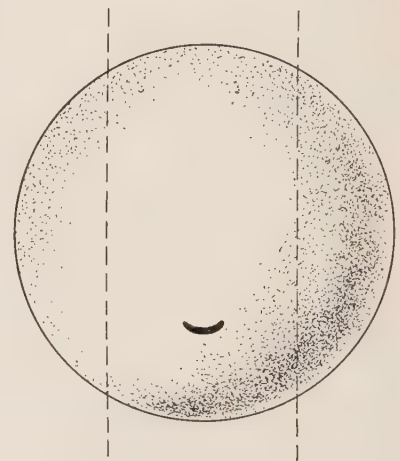


Figure 2a

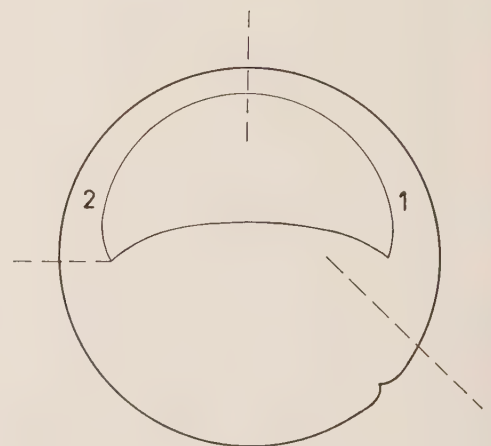


Figure 2b

¹ J. R. GREGG and S. LØVTRUP, C. r. Trav. Lab. Carlsberg, Sér. Ch. 27, 307 (1949).

² F. FRIEDBERG and R. M. EAKIN, J. exp. Zool. 110, 33 (1949). – J. L. SIRLIN, Exper. 11, 112 (1955). – J. L. SIRLIN and C. H. WADDINGTON, Nature 174, 309 (1954).

³ E. URBANI and L. DE CESARI GOROMALDI, Ric. scient. 24, 2364 (1954).

⁴ W. SHUMWAY, Anat. Rec. 78, 139 (1940).

⁵ F. DUSPIVA, Protoplasma 32, 261 (1939).

rotal (TN) and extractable (EN) nitrogen were used as a reference in each set of experiments. Nitrogen was determined by direct Nesslerization after combustion.

Blastula. Our determinations have shown that in the blastula of *Discoglossus* the EN has the following distri-

portion: $51.4 \pm 2.2\%$ in the animal half and $35.6 \pm 4.5\%$ in the vegetal half.

Accordingly, if protease activity in these two regions is calculated on the basis of TN, a slightly higher activity is found in the animal half: $58.1 \pm 3.06\%$ of the total activity or $0.57 \pm 0.024 \mu\text{M}$ of Tyrosin (per 1 mg N) for the animal half as compared with $0.40 \pm 0.026 \mu\text{M}$ of Tyrosin in the ventral half.

On the basis of EN, on the other hand, it is found that the activity is more or less equally distributed between the two regions, being in the animal half $48.1 \pm 6.4\%$ of the total activity or $1.00 \pm 0.12 \mu\text{M}$ of Tyrosin as compared with $1.20 \pm 0.15 \mu\text{M}$ of Tyrosin in the vegetal half.

Gastrula. The Figures given by GREGG and LØVTRUP¹ indicate that there is no difference in yolk content between region 1 and 2 of the early gastrula. Our determinations have entirely confirmed this. On the other hand the difference in protease activity between these two regions is striking, being considerably higher in 1 than in 2, respectively of the reference being TN or EN (Table).

Protease activity in regions 1 and 2 of the early gastrula of *Discoglossus pictus*. Activity in micromoles of Tyrosin/1 mg N/2 h incubation at 30°C.

Experiment	Activity per TN		Activity per EN	
	Region 1	Region 2	Region 1	Region 2
1/3	0.70	0.14	1.57	0.31
12/3	0.33	0.23	0.48	0.31
19/3	0.64	0.26	—	—
22/3	0.39	0.27	—	—
15/5	0.95	0.22	1.25	0.83
29/5	0.86	0.56	1.29	0.95

These results, although of preliminary character, appear to be in line with the assumption that the presumptive chorda-mesoderm, together with the presumptive neurectoderm of the early gastrula are the site of a higher renewal of proteins.

The activities we have observed are, however, rather low; which has prevented us, thus far, from carrying on the investigation on smaller explants, i.e. on less heterogeneous presumptive regions.

This investigation has been supported by a Grant from Consiglio Nazionale delle Ricerche to the Laboratory of Comparative Anatomy.

V. D'AMELIO and M. P. CEAS

Laboratory of Comparative Anatomy, The University of Palermo, Italy, December 27, 1956.

Riassunto

È stata determinata l'attività proteasica nelle differenti regioni della blastula e della gastrula di *Discoglossus pictus*. Nella blastula non esiste una differenza di attività fra metà animale e vegetativa, quando si prenda come riferimento l'azoto citoplasmatico. Nella giovane gastrula il territorio presuntivo della corda e del sistema nervoso mostra una attività più elevata della epidermide presuntiva.

On the Biological Function of Cathepsin in Tail Tissue of *Xenopus* Larvae

In conclusion from earlier experiments on catheptic activity in larval tails of *Xenopus* during normal and chemically suppressed regeneration, the hypothesis was put forward that cathepsin might be concerned mainly with protein degradation¹. However, in literature which refers to embryonic development² or to regeneration³, both synthetic and catabolic functions are assigned to cathepsin. Obviously breakdown of proteins occurs during early phases of regeneration and in embryos, when yolk is utilised, but always concomitantly with formation of new tissues. Hence it appears rather difficult to draw definite conclusions on the predominating function of the cathepsin system.

During development, the tail of *Anuran* larvae shows a clear sequence of morphogenetic events in which growth is followed by tissue resorption. At the chemical level, this would involve first mainly synthesis and later breakdown of tissue proteins. Hence a comparison of catheptic activity during these phases would permit more precise conclusions on its functional significance.

Xenopus larvae were selected after hatching and raised in small groups at 20°C under optimal feeding conditions. At the onset of metamorphosis (i.e. eruption of the fore limbs⁴) the larvae were transferred individually into glass-distilled water and kept without food, until they were used. For staging, the anaesthetised larvae (MS 222 1:6000) were measured ventrally. 'Body length' is the distance from the upper jaw to the tail tip, and 'tail length' is defined by the distance from the caudal intersection between the hind limb and the tail to the tail tip. These readings are accurate to within ± 0.1 mm.

Catheptic activity was determined according to the method of DUSPIVA⁵, which uses casein-urea as substrate. Since details of this procedure are described elsewhere¹, only the modifications for the present experiments are mentioned:

a) Homogenates of tails, amputated 1 mm behind the anus, were prepared in glass-distilled water at low temperature ($+ 2^\circ\text{C}$).

b) Catheptic activity was expressed in terms of an arbitrarily fixed 'Tail Unit' by using a standard curve which relates extent of splitting to homogenate (i.e. cathepsin) concentration. These data were based on corresponding quantities of total nitrogen, determined according to the micromethod of BOELL and SHEN⁶. Assays for catheptic activity ($\bar{x}$) and total nitrogen ($\bar{y}$) were done in triplicates of the same homogenate. The standard error for the specific activity ($\bar{x}/\bar{y}$) was calculated according to the following formula⁷:

$$s_{\bar{x}/\bar{y}} = \pm \frac{1}{\bar{y}} \cdot \sqrt{s_{\bar{x}}^2 + \left(\frac{\bar{x}}{\bar{y}}\right)^2 \cdot s_{\bar{y}}^2}$$

Growth and resorption of the larval tail. Body and tail lengths increase at linear rates which fall slightly before

¹ P. K. JENSEN, F. E. LEHMANN, and R. WEBER, *Helv. physiol. Acta* 14, 188 (1956).

² S. LØVTRUP, *C. r. Lab. Carlsberg, sér. chim.* 29, 261 (1955). – E. URBANI, *Exper.* 11, 209 (1955).

³ J. NEEDHAM, *Biochemistry and Morphogenesis* (Cambridge Univ. Press, 1950).

⁴ P. GASCHÉ, *Helv. physiol. Acta* 2, 607 (1944).

⁵ F. DUSPIVA, *Protoplasma* 32, 211 (1939).

⁶ E. J. BOELL and S. C. SHEN, *Exper. Cell. Res.* 7, 147 (1954).

⁷ I am indebted to Prof. S. ROSIN (Bern) who was kind enough to work out this formula.

the first signs of metamorphosis appear (Fig. 1). In the present group of larvae, this happened at an age of 41 (1 larva), 46 (2 larvae) and 47 days (1 larva) respectively,

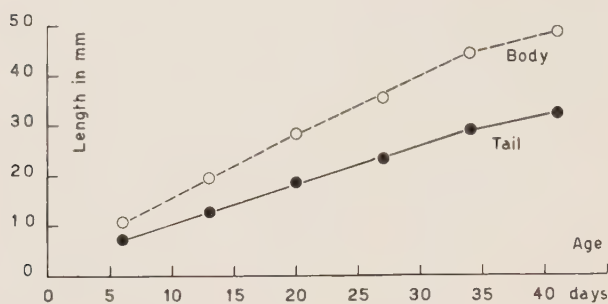


Fig. 1.—Growth curves for body and tail lengths of *Xenopus* larvae. Each point represents the mean of 4 larvae raised at 20°C.

at a mean tail length of 32.3 mm. In contrast to the rather extended growth period, tail resorption occupies a few days only (Fig. 2). The maximum tail length (L_{max}) is attained at the beginning of metamorphosis.

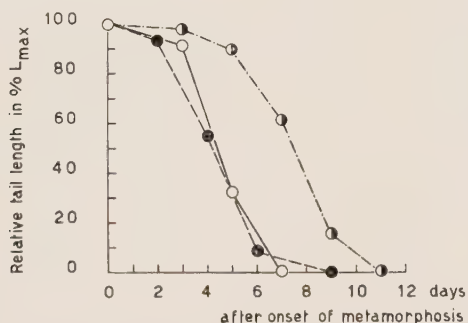


Fig. 2.—Reduction of tail length during metamorphosis. Each curve shows the tail reduction for one larva at 20°C.

Actually, the rate of tail resorption as judged from the decreasing tail length is not constant and extends over varying lengths of time. In all cases, after a slow initial reduction, resorption sets in at a great speed, thus producing a decrease in tail length of about 70% within only four days.

Since at a given temperature growth rate and the beginning of metamorphosis depend upon the state of nutrition⁴, tail length should provide a more reliable reference than age for larval stages.

Catheptic activity during growth and resorption of tails. There is a greater variation in specific activity within a given batch of larvae than between larvae from different batches, but in relation to tail length a general trend of the curve is evident (Fig. 3). If all larvae up to 29 mm tail length are considered, the regression line indicates a falling specific activity of cathepsin in relation to increasing tail length. On the other hand, the regression line, referring to the larvae > 29 mm tail length up to the onset of metamorphosis, demonstrates an increasing trend of the specific activity in pre-metamorphosis larvae. From the t-test, the difference between the mean specific activities for larvae with tail lengths > 15–29 mm and > 29– L_{max} was found to be highly significant ($1\%_{00} > P > 1\%$), thus demonstrating a higher level of catheptic activity in tail tissue of pre-metamorphosis larvae.

During metamorphosis, when the larval tail is resorbed by autolysis, the specific activity shows an almost exponential increase (Fig. 4). In the last resorption

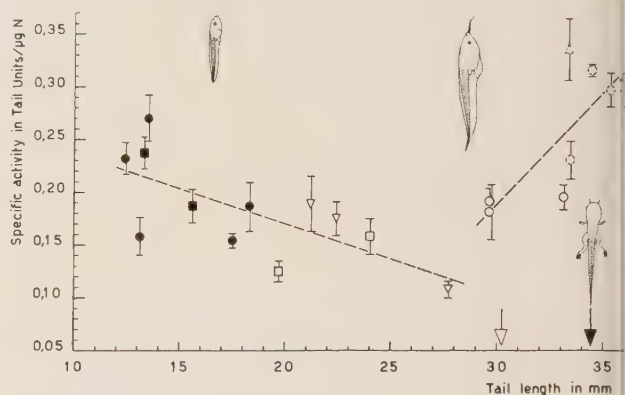


Fig. 3.—Specific catheptic activity during tail growth. Larvae from different batches are indicated by special signs, white ones referring to individual and black ones representing 3 larvae. Signs with broken lines indicate larvae at the beginning of metamorphosis. The black arrow marks the mean maximum length and the white one gives the minimum length of tails, observed in 34 larvae with erupted fore-limbs. The vertical lines represent the standard error of the specific activity.

stages, the activity is about 22 times higher than at the beginning of metamorphosis.

These experiments show: (1) that tail growth (i.e. formation of tissue proteins) is characterised by a decrease in catheptic activity per unit total nitrogen; (2) that this

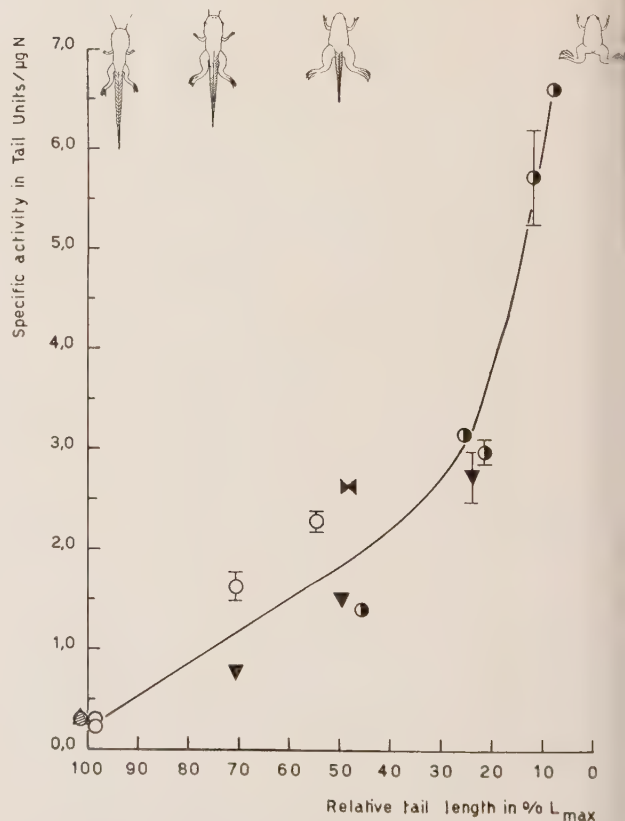


Fig. 4.—Specific catheptic activity during tail resorption. All signs refer to individual larvae; their maximum tail length (L_{max}) was fixed as 100%. The standard error is only indicated in those cases where it exceeds the diameter of the sign.

rend is, however, changed in pre-metamorphosis larvae, when the tails are still growing and as yet no signs of tissue resorption are detectable; (3) that, concomitantly with progressive tail resorption (metamorphosis), a spectacular increase in catheptic activity takes place. Hence, there is good evidence to assign a predominantly catabolic function to the cathepsin system in tail tissue of *Xenopus* larvae. It is not yet known which factors control catheptic activity, but preliminary results, obtained by paper chromatography of methanol extracts of tails, revealed remarkably different patterns of amino acids and peptides in growing and resorption stages. Finally, the high level of activity observed in pre-metamorphosis larvae could reflect a physiological change in the tissue. It may have some relation to the onset of 'competence'³ in the tail tissue, i.e. its reactivity to metamorphosis stimuli. This hypothesis, however, remains to be confirmed by further experiments.

This work was supported by grants from the 'Swiss National Fund'.

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December 21, 1956.

Zusammenfassung

Die Bestimmung der Kathepsinaktivität an wachsenden und in Rückbildung begriffenen Schwänzen von *Xenopus*larven mittels der von DUSPIVA⁵ ausgearbeiteten Casein-Harnstoffmethode führte zur Feststellung, dass dem Kathepsinsystem eine vorwiegend proteolytische Funktion zukommt.

Anoxygene Reaktivierung der Zellvermehrung nach anoxybiotisch hervorgerufener Zytostase (Anabiose)

Aerob ausschliesslich atmende Zellen (Atmungszellen) sind ausserstande, sich ohne die Mitwirkung von Sauerstoff fortzupflanzen¹. Aerob gärende Zellen dagegen (Gärungszellen) bleiben im O₂-freien Substrat noch über eine Reihe von Passagen partiell vermehrungsfähig, verlieren aber von Generation zu Generation sukzessiv an Proliferationskraft, bis sie gänzlich zu wachsen aufhören (Anabiose)². Dass es nicht die Gärung ist, welche die Energie für das partielle Wachstum in der Anoxybiose liefert, geht experimentell daraus hervor, dass die unabiotischen Zellen unbeeinträchtigt weitergären³, wobei auch nicht ein Substratwechsel (Entfernung der Gärungshemmstoffe, erneute Zuführung von Akzessorien) die Zytostase aufzuheben vermag. Erst wenn dem System wieder Sauerstoff zugeleitet wird, tritt reversibel Zellvermehrung ein und später sogar, nach kontinuierlicher O₂-Einwirkung, partielle Proliferation unter anaeroben Bedingungen.

Der in der Anoxybiose diminutiv verlaufende Wachstumsprozess der Gärungszellen und die oxydative Re-

aktivierbarkeit ihrer anaeroben Proliferationsfähigkeit nach eingetretener Anabiose sind nicht anders zu erklären als durch das Vorhandensein eines anoxygenen Energiepotentials, dessen respiratorische Entstehung und Akkumulation, vom unabiotischen Zellstadium ausgehend, sich auch tatsächlich auf chromoanalytischem Wege nachweisen lässt⁴. Evidenter noch tritt – mit der Testschärfe des von uns ausgearbeiteten histochemischen M-T-Verfahrens⁵ – das anoxygene Energiepotential bei bestimmten zytoplasmatischen Reaktionen des mikrobiellen Phosphatwechsels in Erscheinung⁶.

Auf der experimentellen Suche nach dem Energie-reservestoff – der in der Anoxybiose synthetische Zellreaktionen (endergonisch) ermöglicht, im Verlaufe der Diminutionszüchtung aufgezehrt und bei darauffolgender Oxybiose erneut gebildet wird – leiteten wir die entsprechenden Isolierungsmassnahmen in der Weise ein, dass wir aus den normalen Gärungs- und Atmungszellen nach bekannter Methode⁷ Zellkochsäfte herstellten. Diese liessen wir alsdann, unter Wahrung strengster (doppelt gesicherter) anaerober Versuchsbedingungen, auf unabiotisches Zellmaterial in vermehrungsfähiger Aussaat einwirken.

In den als Beleg angeführten Fällen (Tabelle) erfolgte die Bereitung der Zellkochsäfte aus *Saccharomyces carlsbergensis* Rasse U, *Torulopsis* Stamm S und *Candida Mycoderma* (REESS). Zur Kontrolle wurden in jeder Gruppe Proben mit Malzwürze (10%ig) vorgenommen. Als Testzellen dienten gleichfalls die oben angegebenen drei Hefearten, die erstere bis zur Anabiose diminutiv gezüchtet, die beiden folgenden in desoxygenem Zustand verwendet. Sämtliche Zellkochsäfte gelangten im Verschnitt 1:1 mit 15%iger Vorderwürze zum anoxybiotischen Ansatz. Die kritischen Proliferationsprüfungen führten wir – unter galvanometrischer O₂-Spurenkontrolle nach TÖDT⁸ – in einer hermetisch abgeschlossenen Anoxybiose-Apparatur⁹ durch. Während der zwischenzeitlichen Bebrütungsstadien waren die Kultivierungskolben in gasdicht isolierten, mit Reinstickstoff beschickten Sammelbehältern abgestellt. Das entscheidende generative Ergebnis, um das es sich vorliegend zunächst handelt, zeichnet sich beim Vergleich der charakteristischen Proliferationsdaten (Tabelle) zwischen den einzelnen Gruppen und innerhalb jeder Zellkategorie mit aller Deutlichkeit ab.

Wertet man in umstehender Tabelle die erste Gruppe (*Sacch. carlsbergensis* Rasse U) zunächst für sich allein aus, so leitet sich – auf Grund der experimentellen Endvermehrungszahlen – der Nachweis daraus ab, dass im Zellkochsaft der Gärungs- und Atmungszellen ein thermostabiler Reserveenergiestoff enthalten ist, der unter anoxygenen Bedingungen die Zellvermehrung nach eingetretener Anabiose zu reaktivieren oder, biodynamisch ausgedrückt, die respiratorische Funktion der ergonischen Reaktionskoppelung zu substituieren vermag. Im

⁴ F. WINDISCH, W. NORDHEIM und W. HEUMANN, Z. physiol. Chem. 303, 153 (1956).

⁵ F. WINDISCH und D. STIERAND, sowie H. HAEHN, Protoplasma 42, 346 (1953).

⁶ F. WINDISCH, W. HEUMANN und W. NORDHEIM, Naturwissenschaften 42, 649 (1955). – F. WINDISCH und W. NORDHEIM, Biol. Zbl. 1956 (im Druck).

⁷ H. HAEHN, Biochemie der Gärungen (De Gruyter, Berlin 1952), S. 403.

⁸ F. TÖDT, Z. Elektrochem. 54, 485 (1950). – Gemeinschaftsarbeit von F. WINDISCH, W. HEUMANN und CHR. GOSLICH, sowie F. TÖDT und G. TESKE, Biochem. Z. 323, 192 (1952).

⁹ F. WINDISCH, H. HAEHN und W. HEUMANN, Arch. Geschwulstforsch. 6, 64 (1953), und zwar S. 67–69.

¹ F. WINDISCH, W. HEUMANN und CHR. GOSLICH, Z. Naturforsch. 8b, 305 (1953).

² F. WINDISCH, H. HAEHN und W. HEUMANN, Arch. Geschwulstforsch. 6, 64 (1953).

³ F. WINDISCH, W. HEUMANN und CHR. GOSLICH, Biol. Zbl. 74, 1956 (1955).

Vergleich der Endvermehrungszahlen von anabiotischen Atmungs- und Gärungshefen nach anoxygener Überimpfung in Malzwürze bzw. Zellkochsaft + Malzwürze (1:1)

Testzellen	Verwendetes Substrat	Zellenzahl/mm ³	
		Aussaatmenge	Endzellenzahl
<i>Saccharomyces carlsbergensis</i> Rasse U (anabiotisch) $Q_{CO_2}^{N_2} = 280$	Zellkochsaft aus <i>Saccharomyces carlsbergensis</i> U + Malzwürze	500	24 000
	Zellkochsaft aus <i>Torulopsis</i> S + Malzwürze	200	14 500
	Zellkochsaft aus <i>Candida Mycoderma</i> + Malzwürze	300	20 500
	10%ige Malzwürze	500	unverändert
<i>Torulopsis</i> Stamm S $Q_{CO_2}^{O_2} : Q_{O_2} \approx 1$	Zellkochsaft aus <i>Saccharomyces carlsbergensis</i> U + Malzwürze	4000	unverändert
	10%ige Malzwürze	5000	unverändert
<i>Candida Mycoderma</i> (REESS) $Q_{CO_2}^{O_2} : Q_{O_2} \approx 1$	Zellkochsaft aus <i>Saccharomyces carlsbergensis</i> U + Malzwürze	2000	unverändert
	Zellkochsaft aus <i>Candida Mycoderma</i> + Malzwürze	2500	unverändert
	10%ige Malzwürze	6000	unverändert

Vergleich mit den übrigen Gruppen (*Torulopsis* Stamm S, *Candida Mycoderma*) zeigt es sich demgegenüber, dass der in allen untersuchten Zellkochsäften nachgewiesene Reserveenergiestoff *nur* in Kontakt mit den Gärungszellen, *nicht* aber mit den Atmungszellen eine feststellbare generative Wirkung ausübt. Hieraus gibt sich zugleich zu erkennen, dass dem letzteren Zelltypus (aerob ausschliesslich atmend) ein aktivierender Co-Faktor fehlt, der offensichtlich nur dem aerob gärenden Zelltypus eigen ist¹⁰. Es besteht also ein deutlicher Zusammenhang zwischen aerobem Gärungsvermögen und Aktivierbarkeit des Reserveenergiestoffes in generativer Hinsicht, obwohl andererseits die Zuckerspaltung, was hier noch einmal besonders hervorgehoben werden soll, ergonisch *nicht* dazu imstande ist, das Zellwachstum unter anaeroben Bedingungen aufrechtzuerhalten bzw. nach erfolgter Sistierung zu reaktivieren¹¹.

Mit analoger Methodik konnten wir in Zellkochsäften aus dem Jensen-Sarkom der Ratte und anderen Krebsgeweben einen gleichartig wirkenden Reserveenergiestoff nachweisen. Diesen für die Krebsforschung bedeutungsvollen und weittragenden Befund möchten wir hier vorerst dokumentieren, um in nachfolgenden Arbeiten die experimentellen Belege zu geben und eingehen-der darüber zu berichten.

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Institut für Medizin und Biologie, Deutsche Akademie der Wissenschaften, Berlin, den 8. Dezember 1956.

Summary

In cell juice of aerobic fermenting and of only respiring yeast cells, a thermostable respiratory energy

reserve is found which under rigorous anoxybiotic conditions effects a generative reactivation in contact with anabiotic fermenting yeast cells, but *not* with only respiring ones. Also in cell juice of Jensen sarcoma and other carcinoma tissues, an adequate energy substance is recognized as being able to replace oxygen biologically.

Le transaminasi negli stati di deficienza di cocarbossilasi

In precedenti ricerche avevo dimostrato, in condizioni (avitaminosi C) di ridotta utilizzazione di chetoacidi transaminabili¹ (ac. piruvico, ac. α -chetoglutarico), un aumento della glutammico piruvico e della glutammico ossalacetico transaminasi, cioè di quegli enzimi che presiedono al metabolismo di dette sostanze per via transaminativa. Ed essendo tale aumento direttamente proporzionale alla aumentata concentrazione dei due chetoacidi, e alla diminuzione della cocarbossilasi, ho formulato l'ipotesi che la transaminasi possa avere funzione vicariante nei confronti della cocarbossilasi; e cioè, ogni qualvolta per diminuita attività cocarbossilasica si verifichi aumento di chetoacidi, essa intervenga a trasformare questi ultimi in composti (i corrispondenti, aminoacidi) a dissociazione acida minore, proteggendo in tal modo la riserva alcalina dell'organismo compromessa. Onde controllare l'attendibilità di questa ipotesi, ho esteso lo studio delle transaminasi ad altri stati di α -chetoacidosi. Ho scelto il diabete allossanico, in quanto, in queste condizioni sperimentali, si verifica un quadro analogo a quello già da me dimostrato nell'avitaminosi C: e cioè una aumentata concentrazione di acido piru-

¹⁰ F. WINDISCH, W. NORDHEIM und W. HEUMANN, Arch. Mikrobiol. 1956 (im Druck).

¹¹ F. WINDISCH, W. HEUMANN und CHR. GOSLICH, Biol. Zbl. 74, 646 (1955), und zwar S. 656-660.

¹ E. BARBIERI, La Ricerca Scientifica, Supplemento anno 25, 325 (1955); Riv. Biol. 1956 (in corso di stampa); Atti Istituto Veneto Sci. Lett. Arti 112, 159 (1954); Arch. Sci. Biol. 39, 443 (1955).

vico e α -chetoglutarico² con diminuzione dell'attività cocarbossilasica³.

Ratti albini del peso medio di g 250 erano trattati con allossana per via endoperitoneale (mg 150/kg) e sacrificati circa 10 giorni dal trattamento, dopo aver controllato, a digiuno da 12 h, il contenuto ematico di glucosio⁴, acido piruvico e α -chetoglutarico⁵.

Negli organi rapidamente prelevati e messi in bicchiere contenente H₂O e ghiaccio, si dosava quindi la glutammico piruvico e la glutammico ossalacetico transaminasi (secondo il metodo descritto in una precedente nota⁶, e seguendo il decorso da sinistra verso destra delle seguenti reazioni: glutammico + piruvico $\rightleftharpoons$ α -chetoglutarico + alanina; aspartico + α -chetoglutarico $\rightleftharpoons$ ossalacetico + glutammico).

I risultati si possono così riassumere:

a) I due chetoacidi transaminabili (piruvico e chetoglutarico) sono aumentati nel diabetico (ratto normale: ac. piruvico mg 1,6%; ac. α -chetoglutarico mg 0,64%; ratto diabetico: ac. piruvico mg 2,7%; ac. α -chetoglutarico mg 0,95%). Il loro aumento è proporzionale all'intensità dell'iperglicemia e al grado di diminuzione di cocarbossilasi.

b) Anche le transaminasi del fegato sono aumentate e il loro aumento è proporzionale a quello dei due chetoacidi sui quali si svolge la loro azione (ratto normale: glutammico piruvico transaminasi 28,5 μ l di ac. piruvico scomparsi per mg peso secco; glutammico ossalacetico transaminasi 320 μ l di ac. ossalacetico formati per mg peso secco. Ratto diabetico: glut. piruvico 47,2 μ l di ac. piruvico scomparsi per mg tessuto secco; glut. ossalacetico 650 μ l di ac. ossalacetico formati per mg tessuto secco).

Per controllare se l'aumento della transaminasi possa interpretarsi come un tentativo da parte dell'organismo di difendersi dalla minaccia dell'acidosi, ho trattato per 5 giorni un lotto di ratti diabetici con fruttosio (g 1 pro kg per via endoperitoneale): avevo precedentemente dimostrato⁷ che il fruttosio normalizza nello scorbutico e migliora nel soggetto normale l'attività cocarbossilasica, migliorando pure la utilizzazione del glucosio, del piruvato, del chetoglutarato e del lattato; inoltre, come migliora nello scorbutico l'utilizzazione dei chetoacidi attraverso la cocarbossilasi, ritornava alla norma anche l'aumentata attività degli enzimi transaminanti i chetoacidi stessi. D'altra parte anche nel diabetico allossanico si ha normalizzazione dell'attività cocarbossilasica mediante somministrazione di fruttosio⁸.

Ne è risultato che nel diabetico allossanico, la somministrazione di fruttosio normalizza sia la concentrazione dei due chetoacidi nel sangue, sia la attività transaminasica epatica.

Questi risultati depongono a favore della ipotesi precedentemente da me formulata e cioè dell'intervento delle transaminasi nel tentativo di proteggere l'organismo dallo stato di α -chetoadidosi; essi inoltre fanno assumere

a tali enzimi particolare importanza nella regolazione dell'equilibrio acido-base dell'organismo.

E. BARBIERI

Istituto di Chimica Biologica dell'Università di Padova, il 21 dicembre 1956.

Summary

Pyruvic and α -ketoglutaric acid transaminase is increased in α -ketoacidosis caused in guinea pigs and rats by cocarboxylase deficiency (avitaminosis C, alloxan diabetes). Fructose administration not only normalizes the increased concentration of ketoacids and the decreased cocarboxylase activity, but also the increased amount of transaminase. It is suggested that in α -ketoacidosis from transaminable ketoacids the increased transaminating activity might be substitute for reduced cocarboxylase activity.

The Conversion of Ethanolamine into Taurine

The cleavage of S-aminoethylcysteine to cysteamine by the rat has been supported by the detection of cystamine in the urine of the rat injected with synthetic aminoethylcysteine¹. Although cystine, when given in large amounts, produces an excretion of cystamine², cysteine injected in the same molar quantity as aminoethylcysteine did not produce any appreciable increase in non-cystine disulfide-sulfur¹. This finding indicates that, in the case of aminoethylcysteine, the carbon skeleton of the recovered cystamine is likely to come from the aminoethyl moiety of the injected compound.

The biological cleavage of aminoethylcysteine to cysteamine cannot be taken as evidence for the occurrence of a transulfurative reaction from cysteine to ethanolamine unless aminoethylcysteine is found in biological material or the investigation with labelled compounds gives an unequivocal answer. The enzymatic cleavage of a number of thioethers of cysteine was in fact found to take place³, although the reaction does not appear to have any biological significance.

In order to test the passage of the carbon of ethanolamine into that of cysteamine, a mouse was injected with C¹⁴ labelled ethanolamine and the radioactivity of the carbon of cystine and taurine extracted from the animal was analyzed. Cystamine was also isolated from the same animal, but the yield was very poor and the sample was not suitable for analysis. Nevertheless the comparative analysis of cystine and taurine extracted after the injection of ethanolamine gave the desired information. Both cystine and cysteamine are converted by the animal into taurine, and, in the case of the passage of ethanolamine into taurine through cysteamine, the isolated cystine should have given a lower specific activity than taurine.

C¹⁴ uniformly labelled ethanolamine was obtained according to the method of PILGERAM *et al.*⁴, starting

² C. E. FROHMAN, J. M. ORTEN e A. H. SMITH, J. biol. Chem. 193, 803 (1951). - A. FIDANZA e F. LAVIANO, Boll. Soc. Ital. Biol. Sper. 27, 1468 (1951).

³ D. SILIPRANDI e N. SILIPRANDI, Acta Vitaminolog. 5, 1 (1951).

⁴ N. NELSON, J. biol. Chem. 153, 375 (1944).

⁵ J. W. GOODWIN e C. R. WILLIAMS, Biochem. J. 51, 708 (1952).

⁶ E. BARBIERI, La Ricerca Scientifica, Supplemento anno 25, 319 (1955).

⁷ E. BARBIERI, Boll. Soc. Ital. Biol. Sper. 32, 396, 398 (1956); Atti Congr. Biol. Sper., Pavia 1956 (in corso di stampa).

⁸ C. R. ROSSI, F. ROSSI e C. S. ROSSI, Exper. 12, 389 (1956).

¹ D. CAVALLINI, B. MONDOVI, and C. DE MARCO, Biochim. biophys. Acta 18, 122 (1955).

² D. CAVALLINI, C. DE MARCO, and B. MONDOVI, Ric. Scient. 25, 2901 (1955).

³ F. BINLEY, J. biol. Chem. 186, 287 (1950).

⁴ L. O. PILGERAM, E. M. GAL, E. N. SASSENATH, and D. M. GREENBERG, J. biol. Chem. 204, 367 (1953).

from 0.5 mc uniformly labelled commercial ethylene oxide (Tracerlab). A total of 0.67 mM of C^{14} ethanolamine, with 5.2×10^7 c/min/mM was injected in three portions at 24 h interval into a 20 g mouse. The animal was then killed, the skin removed, and homogenized in a Waring blender with 70 ml water. The mixture was boiled for 30 min and filtered. The filtrate was used for the isolation of taurine and the insoluble coagulate was used for the extraction of cystine after hydrolysis in 3 vol. 6 N HCl. Taurine was isolated by a procedure similar to that described by VERLY *et al.*⁵, using 50 mg of carrier. The final crystalline product was checked for purity by chromatography and it was recrystallized 6 times from water-ethanol to constant radioactivity. Cystine was obtained by the cuprous mercaptide precipitation and recrystallized 5 times at the isoelectric point to constant radioactivity. Purity was checked by chromatography and by the SULLIVAN method⁶. Radioactivity was measured after conversion of the compounds to $BaCO_3$ and correcting for self-absorption. The results are reported in the table.

Specific activity of injected ethanolamine and the extracted taurine and cystine

	c/min/mM
Ethanolamine	3.5×10^7
Cystine	6.6×10^4
Taurine	1.3×10^4

Inspection of the table shows that the mouse converted a small amount of ethanolamine into taurine and that cystine has a higher specific activity than taurine. This finding rules out the possibility that the taurine produced comes directly through: ethanolamine $\rightarrow$ cysteamine $\rightarrow$ taurine, in which case cystine, being excluded from the path, should not have been labelled. The results indicate that the route was probably: ethanolamine $\rightarrow$ glycine $\rightarrow$ serine $\rightarrow$ cystathionine $\rightarrow$ cysteine $\rightarrow$ taurine. All the steps of the last-mentioned route have in fact been found to be of biological occurrence⁷.

As a result of the present work, it may be concluded that the transulfuration of ethanolamine is a process of secondary importance, if it exists at all. Thus the mechanism of the biosynthesis of cystamine represents a problem which still requires a solution.

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Institute of Biological Chemistry of the University, Departments of Biochemistry and Biology of the Istituto Superiore di Sanità, Rome, December 27, 1956.

Riassunto

L'etanolamina marcata con C^{14} è trasformata nel topo in taurina e cistina. La cistina ha una attività specifica più elevata della taurina. Questa constatazione indica che la transulfurazione della etanolamina nel topo è un processo biologico di scarsa importanza.

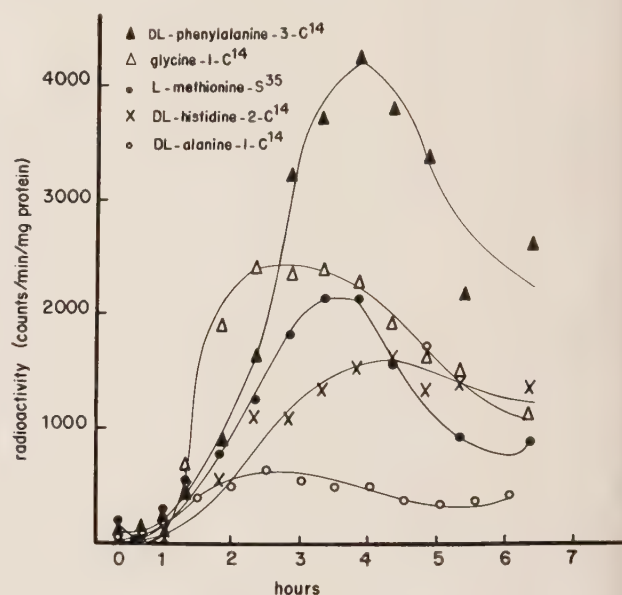
⁵ W. G. VERLY and G. KOCH, *Biochem. J.* **58**, 663 (1954).

⁶ M. X. SULLIVAN and W. C. HESS, *J. biol. Chem.* **116**, 221 (1936).

⁷ A. WEISSBACH and D. B. S. SPRINSON, *J. biol. Chem.* **203**, 1031 (1953). - D. SHERMAN, *J. biol. Chem.* **162**, 297 (1946). - V. DU VIGNEAUD, *A Trail of Research* (Cornell University Press, 1953).

Radioactive Amino Acid Incorporation into the Rat Pancreatic Juice Proteins*

In a previous paper¹ results were presented studying the rate of incorporation of glycine- C^{14} into the rat pancreatic juice proteins. The data obtained suggest that these proteins are built up from blood amino acids and not from preexisting plasma proteins. As our results are based on observations performed only with glycine- C^{14} , it is desirable to extend them to other amino acids. In this paper we present the results obtained utilizing radioactive phenylalanine, alanine, histidine and methionine besides glycine.



Incorporation of radioactive amino acids into the rat pancreatic proteins. Abscissa: Time after injection of radioactive amino acid.

Each point is the average of three animals.

Material and methods. Adult male Wistar rats weighing from 300 to 400 g were used throughout. In the glycine experiment the weight of the animals was 240 g. The anesthesia, stimulation of pancreatic secretion and collection of pancreatic juice was performed as already described². Endovenous injection of 3.3 μ C of the following amino acids was performed 5 min after the secretin-carbamoylcholine stimulus: DL-phenylalanine-3- C^{14} , DL-alanine-1- C^{14} , DL-histidine-2- C^{14} , glycine-1- C^{14} and L-methionine- S^{35} . Three rats were injected with each of the amino acids. 5 μ l samples of pancreatic juice were collected every 20 min during the first 2 h and every half hour for the next 4 h. These samples were spread on stainless steel planchets and their radioactivity measured with a thin mica window Geiger-Müller counter (TGC-2 of Tracerlab). Under these conditions no selfabsorption is observed. The protein content of each planchet was determined by the method described by LOWRY *et al.*³.

* Aided partially by grants from the 'Conselho Nacional de Pesquisas' and 'Deutsche Forschungsgemeinschaft'.

¹ L. C. U. JUNQUEIRA, G. C. HIRSCH, and H. A. ROTHCHILD, *Biochem. J.* **61**, 275 (1955).

² L. C. U. JUNQUEIRA, G. C. HIRSCH, and H. A. ROTHCHILD, *Biochem. J.* **61**, 275 (1955). - G. C. HIRSCH, L. C. U. JUNQUEIRA, H. A. ROTHCHILD, and S. R. DOHR, *Pflügers Arch. ges. Physiol.* (1956) (in press).

³ O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL, *J. biol. Chem.* **193**, 265 (1951).

Results and discussion. The results obtained are presented in the figure. One may see that incorporation of the different amino acids used is similar. Very little radioactivity is present in the first hour after the amino acid injection and the maximum occurs between 2 and 4 h. The incorporation of alanine and glycine presents its maximum around 2½ h, while for histidine this maximum is around 4 h. The maximal incorporation of methionine and phenylalanine is between those two values, i.e. 3½ h. No significance can be attributed to these differences in time owing to the variability observed from animal to animal, as already described in the experiment with glycine¹.

It is difficult to compare the results regarding the specific activity of the samples of pancreatic juice proteins obtained with the different amino acids injected. Although the same amount of μC was injected in all animals, only half of this activity is available to the cell in the case of the DL-amino acids. Even so, wide discrepancies are observed, probably due to the different content of the several amino acids and their conversion products in the proteins studied.

The data above presented suggest that the results and conclusions obtained working with glycine may be extended to the other four amino acids, i.e. that the rat pancreatic juice proteins are synthesized from the free amino acids of the blood.

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Laboratory for Cell Physiology, Faculdade de Medicina, Universidade de São Paulo, Brasil, November 19, 1956.

Zusammenfassung

Es wurde der zeitliche Verlauf des Einbaus radioaktiver Aminosäuren (Phenylalanin-3- C^{14} , Alanin-1- C^{14} , Histidin-2- C^{14} und Methionin-S³⁵) in die Proteine des Pankreassaftes der Ratte untersucht. Ein Maximum des Einbaus kommt zwischen der zweiten und vierten Stunde nach Injektion der Aminosäuren vor, während in der ersten Stunde praktisch keine radioaktiven Proteine nachgewiesen werden konnten. Die Ergebnisse werden verglichen mit denen früher beschriebener Versuche mit Glyzin- C^{14} .

⁴ Permanent address: Zoologisches Institut, Göttingen.

Versuche zur chromatographischen Trennung der im Augenstein von *Astacus astacus* enthaltenen myotropen Wirkstoffe

Nachdem durch frühere Arbeiten erwiesen war, dass im Augenstein des Flusskrebse (*Astacus astacus*) ein Wirkstoff gebildet wird, der die Bewegungen isolierter Därme von Insekten (Larven von *Tenebrio molitor*)¹ und Säugern (Meerschweinchen und Kaninchen)² positiv myotrop beeinflusst, wurden Versuche unternommen, die aus dem Augenstein gewonnenen Extrakte durch Methoden der Papierchromatographie zu reinigen. Die

in diesem Extrakt enthaltenen Substanzen sollten getrennt werden, um nach Möglichkeit den Wirkstoff zu isolieren und seine chemische Natur näher zu definieren.

Zur Extraktbereitung wurden jeweils 2 Paar Augensterne im Achatmörser zerrieben, in 0,15 ml Aqua destillata aufgenommen und 15 min bei 2400 Touren zentrifugiert. Von dem überstehenden Extrakt wurden jeweils 0,02 ml auf das Papier gebracht. Der Startpunkt war 3 cm vom unteren Rand entfernt.

Zur Entwicklung der Chromatogramme wurde aufsteigend in Rundzylindern von 38 cm lichter Höhe und 9 cm Durchmesser gearbeitet. Als die geeignetste Papiersorte erwies sich S und S 2043 a Gl. Als Lösungsmittel diente ein Gemisch aus Butanol-Essigsäure-Wasser 8:5:2, eine Abwandlung des sogenannten «Patridge-Gemisches»³.

Nachdem die aufgebrachte Lösung gut getrocknet war, wurden die Chromatogramme 10–20 h in die Atmosphäre des Lösungsmittels gebracht. Dann wurde entwickelt. Die Laufzeiten betrugen 16–72 h, die Laufstrecken 15–30 cm. Die Chromatogramme wurden danach getrocknet und die einzelnen Flecke mit Ninhydrin (2% in 95% Butanol + 5% 2*n* Essigsäure) sichtbar gemacht.

Es zeigten sich dann 6 Flecke, deren R_f -Werte bei 0,079, 0,112, 0,174, 0,207, 0,250 und 0,368 liegen. Der erste Fleck hat im Kern eine rosa Färbung, die nach dem Rand zu violett wird. Die übrigen 5 Flecke färben sich violett an.

Nicht nachgewiesen werden konnten in den Chromatogrammen Zucker oder allgemein reduzierende Substanzen (Reagenzien: 1% KMnO_4 + 2% Na_2CO_3 in wässriger Lösung, sowie 1% Anilinphthalat in wassergesättigtem Butanol), Adrenalin und Noradrenalin (Reagenz: 2% 4-Dimethyl-aminobenzaldehyd in Butanol), sowie Cholin und dessen Ester (Reagenz: 0,2% Dipikrylamin in Aceton-Wasser 1:1). Auch freie Sulfhydrylgruppen konnten durch Besprühen mit 5% Nitroprussidnatrium in 5% Essigsäure, die mit Ammoniumsulfat gesättigt ist, und anschließendes Einbringen in eine Atmosphäre von konzentriertem Ammoniak nicht festgestellt werden. Freie Sulfhydrylgruppen färben sich nach dieser Methode leuchtend rot, um später in eine beständige Blaufärbung überzugehen.

Zur Prüfung der Wirksamkeit der einzelnen Substanzen wurden auf 7,5 cm breiten Papierstreifen 6 Chromatogramme nebeneinander entwickelt. Eines wurde abgetrennt und mit Ninhydrin sichtbar gemacht. Nach diesem Leitchromatogramm wurden aus den übrigen 5 Streifen die einzelnen Substanzen 24 h in $n/10$ Salzsäure eluiert. Die Eluate wurden nach dieser Zeit auf dem Wasserbade abgedampft, die Salzsäure abgeraucht und die Rückstände in 1 ml Warmblutringer aufgenommen.

Diese Extrakte wurden am isolierten Meerschweinchenileum untersucht. Hierbei zeigte sich, dass der positiv myotrope Wirkstoff in Zone 1 lokalisiert ist. Das Eluat dieser Zone ruft am isolierten Darm dieselben Bewegungsänderungen hervor (Steigerung von Tonus, Amplitude und Frequenz) wie der rohe Augensteinextrakt². Die Eluate aller anderen Zonen des Chromatogrammes waren ebenso wirkungslos wie die Eluate der durchgeführten Blindversuche.

Es ist somit möglich, die myotrop wirksame Phase von den anhaftenden Begleitstoffen zu trennen und, wenigstens in Lösung, mit dem isolierten Wirkstoff zu arbeiten.

¹ G. KOLLER, Verh. dtsch. Zool. Ges., Tübingen 1954, 18, 417 (1955).

² H. KUHNEN, Verh. dtsch. Zool. Ges., Erlangen 1955, 19, 117 (1956).

³ F. CRAMER, *Papierchromatographie*, 2. Aufl. (Verlag Chemie, Weinheim 1953).

Wie Farbwechselfersuche mit der Sandgarnele (*Crangon crangon*) zeigten, ist der gefundene positiv myotrope Wirkstoff mit dem Farbwechselformon der Crustaceen nicht identisch: Garnelen, die an weissen, schwarzen und mittelhellen Untergrund angepasst waren, wurden Eluate der myotrop wirksamen Zone 1 injiziert. Die Tiere zeigten keine Farbveränderungen.

Zum Vergleich wurden aus Augenstielextrakten von *Crangon* Chromatogramme unter denselben Bedingungen, wie für *Astacus* beschrieben, entwickelt. Diese Chromatogramme sind denjenigen von *Astacus* gleich oder zumindest sehr ähnlich. Sie färben sich mit denselben Reagenzien in gleicher Weise an, und die Substanzen, die bei *Astacus* nicht nachweisbar waren, wurden hier ebenfalls nicht gefunden. Auch die R_F -Werte der einzelnen Zonen scheinen bei beiden Arten übereinzustimmen. Jedoch ist die Anzahl der Versuche, die mit *Crangon* durchgeführt werden konnten, zu gering, um hier bindende Aussagen zu machen.

Um einen weiteren Einblick in die chemische Natur des Wirkstoffes zu gewinnen, wurden Eluate der Zone 1 48 h in 5*N* Salzsäure gekocht und dann in einem 2dimensionalen Chromatogramm entwickelt. Es wurde hierbei nach der Methode von ZAHN³ vorgegangen. Dieses 2dimensionale Chromatogramm zeigte 6 Flecke, deren chemische Konstitution noch nicht näher bestimmt wurde. Jedoch geht aus diesen Versuchen hervor, dass der myotrop wirksame Bestandteil des Augenstielextraktes eine Verbindung ist, die aus mehreren Aminosäuren besteht. Es handelt sich also höchstwahrscheinlich um ein Peptid.

Eine ausführliche Besprechung der Versuche und ihrer Ergebnisse wird an anderer Stelle erfolgen.

G. KOLLER und H. KUHNEN

Zoologisches Institut der Universität des Saarlandes, Saarbrücken, den 19. Dezember 1956.

Summary

The detection of two myotropic substances in the eyestalk and the brain of the crayfish *Astacus astacus* and the shrimp *Crangon crangon* was described². Both substances have an action on the isolated ileum of the guinea pig. The substance extracted from the eyestalk has a positive myotropic one, the substance extracted from the brain an inhibiting one.

Further a method is given for the separation and purification of the positive myotropic substance of the eyestalk by paper-chromatography.

Primary Cancer of the Liver in Rats after Subcutaneous Implantation of Pellets of Progesterone and Testosterone Propionate

The object of this communication is to report the development of primary hepatocellular carcinoma of the liver in castrated female rats implanted subcutaneously with pellets of testosterone propionate and progesterone.

During a recent investigation into the relation of diet to the incidence of spontaneous tumours in our strain of rat, the ovaries of aged female rats, receiving a diet which increased significantly the frequency of uterine

cancers, often contained large corpora lutea, the function of which was not reflected by the state of the vagina or uterus. In view of the presence of a large interstitial body on the ovary, together with the corpora lutea, it was suspected that the uterine and vaginal reactions were the consequence in part of the combined effects of variable amounts of progesterone and of androgen. The possibility was therefore entertained that prolonged stimulation with progesterone and testosterone might in some way be causally related to the increased incidence of uterine cancer observed in our experimental rats. Accordingly, this possibility was tested experimentally.

A group of 11, six-week old female rats, were castrated and immediately thereafter implanted subcutaneously with two pellets, one of progesterone (8 to 10 mg) and one of testosterone propionate (8 to 10 mg). The pellets were prepared in a special mould from the pure, crystalline hormones. As soon as palpation revealed a marked decrease in the size of either pellet, a further pellet was implanted while the rat was under ether anaesthesia. These rats received the same ration as fed our stock animals¹.

Six of the rats died from various causes between the 112th and 543 rd days of the experiment. Apart from reproducing the uterine and vaginal pictures observed in the rats used for the dietary experiments mentioned above, no noteworthy reactions were detected.

In one of the five surviving rats a greatly enlarged liver was palpated on the 696th day after the implantation of the pellets. The animal was killed and a large tumour was found in the left median lobe with multiple secondary nodules in the other lobes. Histological examination disclosed the tumour to be a primary liver cancer of the hepatocellular, trabecular variety. The main portal vein in the hilum contained masses of tumour cells and this was apparently responsible for widespread intrahepatic dissemination. Palpation of the abdomen of the 4 remaining rats disclosed the presence of another liver tumour in one rat which proved on microscopic examination also to be an hepatocellular carcinoma. Macroscopically, this tumour was 2 cm in diameter and was confined to the left lateral lobe. The rest of the liver appeared normal. No tumours were found at laparotomy in the other 3 rats, which are still alive.

The occurrence of two cases of primary liver cancer in this small series of rats is highly significant for the following reasons: (a) CURTIS and BULLOCK² found only 1 benign 'hepatoma' in 2450 rats surveyed during their study of tumours; (b) in 1954 we³ reported our failure to produce liver cancer by methods other than those requiring the use of carcinogens, despite the fact that we had induced many varieties of liver pathology in several hundreds of rats; (c) in a special study of spontaneously-occurring tumours in approximately 1500 rats in our colony we did not find a single case of primary liver cancer; (d) GUERIN⁴ commented on the absence of primary liver cancer in 16,500 rats investigated.

The liver tumours developing in our hormone treated rats were not preceded by cholangio-fibrosis, necrosis or cirrhosis. However, fibrosis did occur between and around the large cancerous nodules. In the same way as fibrosis of the liver can be dissociated from an antecedent

¹ J. GILLMAN, C. GILBERT, and I. SPENCE, *Cancer* 6, 494 (1953).

² F. D. CURTIS and M. R. BULLOCK, *J. Cancer Res.* 14, 1 (1930).

³ J. GILLMAN and C. GILBERT, *Cancer* 7, 1109 (1954).

⁴ M. GUERIN, D. C., *Tumeurs Spontanées des Animaux de Laboratoire* (Amédée Legrand 1954).

fatty change⁵, from necrosis⁶ and from the excessive accumulation of iron⁷ so it would appear now that primary liver cancer, at least in the rat, can also be dissociated from observable necrosis, fibrosis and bile duct hyperplasia. In this connection it should be mentioned that LAWS *et al.*⁸ found that, after feeding aceto-amino-fluorene the neoplastic transformation in ZYMBAL's gland arose from tubules which did not show any antecedent cystic or other pathological change, such as occurs commonly in the liver before the emergence of cancer. It may be too early to assert that the extensive liver disease, accompanied by cirrhosis, commonly seen in the African throughout the Continent of Africa may not necessarily predispose to liver cancer.

Since a malignant neoplasm arising from the epithelium of a respiratory bronchiole and different from that commonly seen in adenosis of the lung was also encountered in one of the rats affected also with liver cancer, it is not unlikely that progesterone and testosterone propionate may be able to induce carcinoma in organs other than the lung and the liver. If the findings can be confirmed in a larger series of rats now under investigation in our laboratory, a different orientation may be given to the attack on the etiology of primary carcinoma of the liver in man.

J. GILLMAN and CHRISTINE GILBERT

C. S. I. R. University Nutrition Research Unit and the Departments of Physiology and Anatomy, University of the Witwatersrand, Johannesburg, South Africa, December 27, 1956.

Résumé

Le carcinome hépatocellulaire du type trabéculaire a paru dans 2 rats sur 5, 745 jours après l'implantation de boulettes de progestérone cristalline et de propionate de testostérone, pesant 8 à 10 mg chacune. Un de ces rats a développé un carcinome provenant de l'épithélium d'une bronchiole respiratoire. L'absence d'une cholangio-fibrose antécédente, d'une nécrose ou d'une cirrhose hors de l'endroit de la tumeur du foie chez les rats soumis aux épreuves expérimentales, peut nécessiter un nouvel examen de la signification des lésions hépatiques comme facteur prédisposant au cancer chez l'homme.

⁵ J. GILLMAN and T. GILLMAN, *Perspectives in Human Malnutrition* (Grune and Stratton, 1951).

⁶ J. GILLMAN, C. GILBERT, and I. SPENCE, *Amer. J. Digest Dis.* 19, 211 (1952). – J. GILLMAN and C. GILBERT, *Ann. N. Y. Acad. Sci.* 57, 737 (1954).

⁷ J. GILLMAN and T. GILLMAN, *Perspectives in Human Malnutrition* (Grune and Stratton, 1951); *Arch. Path.* 40, 239 (1945).

⁸ J. O. LAWS, G. RUDALI, R. ROYER, and P. MABILLE, *Cancer Res.* 15, 139 (1955).

From Parasympathomimetics to Parasympatholytics by Homologous Substitution

The effect of a drug on a biological object is the result of the interaction of drug molecules with receptors.

The activity of a drug is determined by the affinity and the intrinsic activity¹. The affinity is the equilibrium

constant for the interaction of the drug with the receptors, which is in the simplest case equal to the reciprocal of the dissociation constant of the drug-receptor complex. The intrinsic activity (i.a.) is the effect per unit of drug-receptor complex. Drugs with a high intrinsic activity on parasympathetic receptors are pure parasympathomimetics, those with an intrinsic activity zero, are competitive antagonists of the mimetics. Drugs with an intermediate intrinsic activity behave as parasympathomimetics or -lytics, depending on the state of the receptor system. Parasympathomimetics and -lytics have affinity to the same receptor system.

In a physico-chemical sense the reaction between drug and receptor implies an interaction between the electrical fields inherent to their charge-distributions. Not only the field inherent to the kationic and anionic sites and other regions with a high or low electron density, but also those which underly the Van der Waals forces and hydrogen bonds have to be taken into consideration. The affinity is coherent with the interaction between the fields in the most general sense. The intrinsic activity often will be connected with a special part of this interaction.

Two magnitudes are of special importance for the drug-receptor interaction and its biological effect: (a) the charge-distribution on drug and receptors, (b) the sterical configuration of drug and receptor as far as they interfere with the interaction between the fields. Little is known about the molecular properties of the receptors. Of the drug-molecules the chemical structure and certain physico-chemical properties are known.

The study of pharmacodynamics has to be based on the relation between the properties of the drug and the effect. On the basis of the results thus obtained conclusions may possibly be drawn about the properties of the receptors. Evidence is accumulating in the literature² that in homologous series of drugs a change in the structure is often combined with a gradual change in the affinity. In an analogous way the intrinsic activity may be expected to change gradually with the structure.

Most parasympathomimetics contain in their configuration a positive and a negative grouping, located on a certain distance³.

The same holds for the -lytics. This indicates that these groupings are important for the affinity to the parasympathetic receptors. In contrast with the -mimetics, the -lytics have large groups on the 'negative' side of the molecule⁴. With respect to substitution on the positive side of cholinergic drugs, e.g. succinylcholine tested on the myoneural junction, it was found that successive replacing of methyl groups by ethyl groups resulted in a gradual change from mimetic to lytic⁵. On the basis of the considerations given before it may be expected that homologous substitution on the positive, R_3 , or on the negative side, R' , of the dioxolane compound 2249 F (dilvasène)⁶, will result in a gradual change of the

² D. BOVET and F. BOVET-NITTI, *Structure et activité pharmacodynamique des médicaments du système nerveux végétatif* (S. Karger S.A., Bâle 1948). – R. B. BARLOW, *Introduction to chemical pharmacology* (Methuen and Co., Ltd., London 1955).

³ C. C. PFEIFFER, *Science* 107, 94 (1948). – H. R. ING, P. KORDIK, and D. P. WILLIAMS, *Brit. J. Pharmacol.* 7, 103 (1952).

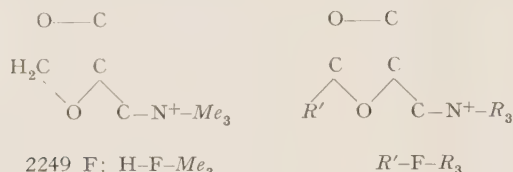
⁴ A. M. LANDS, *J. Pharmacol. exp. Ther.* 102, 219 (1951).

⁵ E. J. ARIËNS, *Arch. int. Pharmacodyn.* 99, 32 (1954). – ST. THESLEFF, K. R. UNNA, *J. Pharmacol. exper. Ther.* 111, 99 (1954). – E. J. ARIËNS, J. M. VAN ROSSUM, and A. M. SIMONIS, *Arzneim.-Forsch.* 6, 282 (1956). – F. v. BRÜCKE, *Pharmacol. Rev.* 8, 256 (1956).

⁶ E. FOURNEAU, D. BOVET, F. BOVET, and J. MONTÉZIN, *Bull. Soc. Chim. Biol.* 26, 134 (1944).

¹ E. J. ARIËNS, *Arch. int. Pharmacodyn.* 99, 32 (1954).

parasymphathomimetic via drugs with a dual mode of action into parasymphatholytics.



1. *Substitution on the 'Negative Side'.*—One hydrogen atom is replaced by an alkyl group increasing in length. It may be expected for this $\text{R}'\text{-F-Me}_3$ series that, starting with H-F-Me_3 , the intrinsic activity decreases, so that finally parasymphatholytics are obtained. The expectation was confirmed by experiments using the rat jejunum as a test object. The drugs H-F-Me_3 , Me-F-Me_3 , and Et-F-Me_3 are parasymphathomimetics (high i.a.). Bu-F-Me_3 and Hex-F-Me_3 are parasymphatholytics (i.a. is zero). (They behave as competitive antagonists of the mimetics Fig. 1). Pr-F-Me_3 with its intermediate intrinsic activity is a drug with a dualism in action. Thus Pr-F-Me_3 behaves as a parasymphathomimetic or a -lytic, dependent on the state of the receptor system (Fig. 2). As may be seen from the table, for all $\text{R}'\text{-F-R}_3$ compounds elongation of the alkyl chain R' results in a decrease of the intrinsic activity.

On the same basis in the $\text{R}_2'\text{-F-R}_3$ series a continuous change from parasymphathomimetics into -lytics may be expected. The experimental confirmation is summarized in the table.

'Intrinsic activities' (i.a.) and log 'affinities'* (aff.)

$\text{R}'\text{-F-R}_3$ compounds								
R'	R_3							
	Me_3		Me_2Et		MeEt_2		Et_3	
	i. a.	aff.	i. a.	aff.	i. a.	aff.	i. a.	aff.
H	1	5.3	1	4.8	0.4	3.6	0.2	3.5
Me	1	7.3	1	7.1	0.3	4.0	0	3.6
Et	1	5.4	0.5	5.0	0	4.6	0	4.2
Pr	0.5	4.9	0	4.9	—	—	0	4.7
Bu	0	4.8	—	—	—	—	—	—
Hex	0	4.7	—	—	—	—	—	—
$\text{R}_2'\text{-F-R}_3$ compounds								
R_2'	i. a.	aff.	i. a.	aff.	i. a.	aff.	i. a.	aff.
Me_2	1	4.9	0.6	4.8	0.2	4.3	0	4.3
Et_2	0.5	4.6	0	4.8	—	—	—	—
Pr_2	0	6.4	0	6.6	—	—	—	—
$\text{R}'\text{-N}^+\text{-R}_3$ compounds								
R'	i. a.	aff.	i. a.	aff.	i. a.	aff.	i. a.	aff.
Bu	1	5.2	0.5	5.1	0	4.0	0	4.3
Pent	1	5.4	0.4	5.2	—	—	0	4.4
Hex	0.9	5.0	0.1	4.7	—	—	0	5.0
Hept	0.1	4.6	0	5.1	—	—	0	5.5
Oct	0	5.0	—	—	—	—	0	5.4
Non	0	5.0	—	—	—	—	0	5.6
Dec	0	5.9	—	—	—	—	0	—
Dodec	0	6.0	—	—	—	—	—	—

acetylcholine: i.a. = 1, aff. 7.0; atropine: i.a. = 0, aff. 8.7

* The log affinity is the pD_2 value for the mimetics and the pA_2 value for the lytics.

2. *Substitution on the 'Positive Side'.*—The methyl groups on the quaternary N-atom are replaced successively by ethyl groups. It may be expected that starting with the parasymphathomimetic Me-F-Me_3 , the intrinsic activity decreases, so that here also parasymphatholytics may be obtained in the end. The experiments confirmed the expectations. Thus it was found that Me-F-Me_3 and $\text{Me-F-Me}_2\text{Et}$ are parasymphathomimetics (high i.a.), Me-F-Et_3 is a -lytic (i.a. is zero).

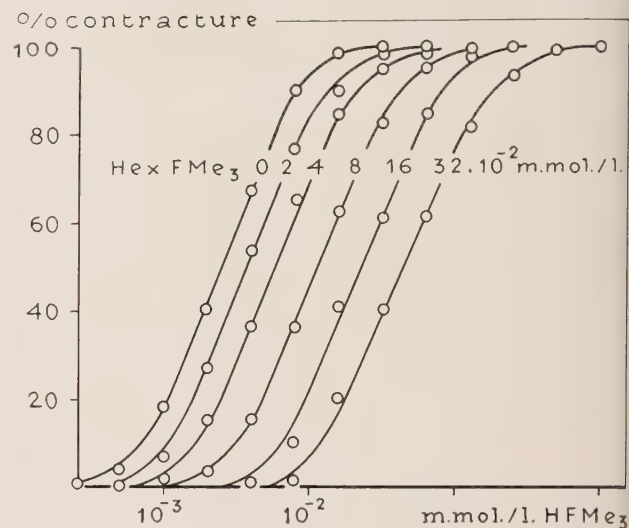


Fig. 1.—Cumulative log dose-response curves on the isolated rat jejunum for H-F-Me_3 in the presence of constant concentrations of the competitive antagonist Hex-F-Me_3 .

Me-F-MeEt_2 is a drug with dualism in action (intermediate i.a.). (See the table.) Successive substitution on the positive side alone is sufficient to change the $\text{R}'\text{-F-R}_3$ compounds from parasymphathomimetics into -lytics.

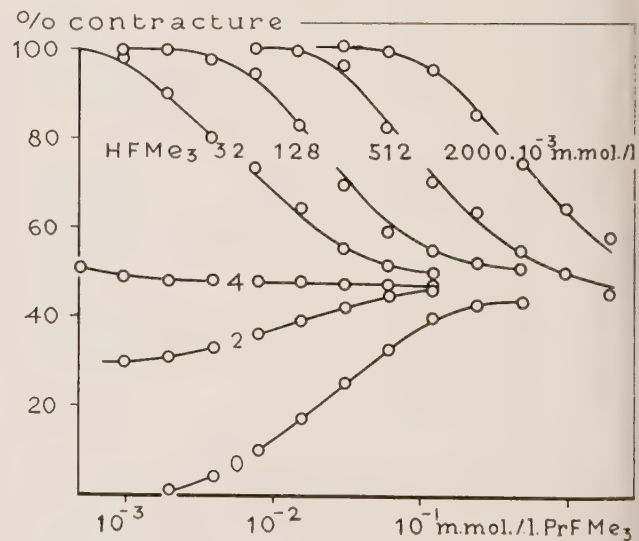


Fig. 2.—Cumulative log dose-response curves on the isolated rat jejunum for Pr-F-Me_3 in the presence of constant concentrations of H-F-Me_3 . Dependent on the response already given with H-F-Me_3 , Pr-F-Me_3 is synergistic, inactive or antagonistic with respect to H-F-Me_3 . Independent of the initial response for high values of Pr-F-Me_3 the same response is always obtained.

Substitution of larger groups on the 'positive' as well as on the 'negative' side results in a change of -mimetic

into -lytic. From a combination of both types of substitution, a more pronounced decrease of the intrinsic activity can be expected. This was confirmed by the experiments. In this way in the various homologous series, the intrinsic activity reached zero for Bu-F-Me₃, Pr-F-Me₂Et, Et-F-MeEt₂ and Me-F-Et₃ (see the table).

Not only for the intrinsic activity but also for the affinity, regular changes with the molecular structure were observed.

It was mentioned before that the charge distribution and the sterical configuration of the drug may be considered to be of special importance for the effect. For drugs with an affinity to cholinergic receptors the most pronounced electrical charge is found on the quaternary N⁺-atom.

Ethylation, which means introduction of larger and electron-repulsing groups, results in a decrease of this positive charge. At the same time as a consequence of sterical hindrance, the influence of this charge on the field of the receptors is decreased. Possibly a kind of masking of the charge by ethyl groups may also be involved. From the experiments it may be concluded that there is a strong correlation between the molecular properties just mentioned and the intrinsic activity. With respect to the affinity, it was found that for the parasympathomimetics ethylation resulted in a decrease of the affinity, while for the -lytics no correlation was found.

Substitution on the negative side has hardly any influence on the charge of the quaternary N⁺-atom. The decrease in the intrinsic activity caused by the elongation of R' probably has to be ascribed to a sterical interference with respect to the approach of the positive charge to the receptors. The negative grouping is not essential with respect to parasympathetic activity on the rat jejunum. This is clearly demonstrated by the results obtained with some series of homologous alkyltrialkylammonium compounds (R'-N⁺-R₃), which closely resemble those obtained with the dioxolane compounds (see table).

Introducing affinity and intrinsic activity into the receptor theory, it was possible to predict biological properties for a large number of new dioxolane compounds and a series of alkyltrialkylammonium compounds.

Acknowledgments. Grateful appreciation is expressed to Misses S. J. M. BRINKHOFF, A. DE MOTS, A. M. MULDER, I. R. M. OBBEMA, and A. M. A. J. PONSIOEN for their valuable technical assistance.

We are indebted to SPECIA, Paris, for the supply of Dilvasène (H-F-Me₃).

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Zusammenfassung

Auf Grund der theoretischen Befunde war zu erwarten, dass Änderungen an der negativen und positiven Seite der Dioxolan-Verbindung 2249 F zu graduellen Übergängen von Parasympathikomimetika zu Parasympathikolytika führen würden. Die Experimente bestätigten die Erwartungen vollauf.

Untersuchung homologer Reihen von Alkyltrialkylammonium-Verbindungen zeigte die negative Gruppe als nicht wesentlich für die parasympathische Wirkung und ergab ebenso einen graduellen Übergang von Mimetika zu Lytika.

Einfluss von Isonikotinsäurehydraziden auf den Verlauf der Körpertemperatur nach Reserpin, Monoaminen und Chlorpromazin

Reserpin, das bei Kaninchen Sedation und Miosis verursacht, bewirkt nach Vorbehandlung mit Isopropylisonikotinsäurehydrazid (Iproniazid, *Marsilid*) Exzitation, Mydriasis und Pilo-Erektion, ähnlich wie Lysergsäure-diäthylamid (LSD)¹. Reserpin setzt gewebegebundenes 5-Hydroxytryptamin (5HT) und Catecholamine in Gehirn und anderen Organen frei². Diese Amine werden zum Teil durch Wirkung von Monoaminoxidase inaktiviert, einem Ferment, das durch Iproniazid und in viel schwächerem Masse durch Isonikotinsäurehydrazid (Isoniazid, *Rimiform*) gehemmt wird³. Die Abbauehemmung der infolge Reserpin-Wirkung freigesetzten Monoamine durch Iproniazid führt möglicherweise zu unphysiologisch hoher Konzentration von freien Monoaminen im Gehirn. Diese Hypothese könnte den LSD-artigen Effekt von Reserpin nach Iproniazid-Vorbehandlung erklären⁴.

In der vorliegenden Arbeit sollte die Bedeutung der Monoaminoxidase bei der Wirkung von Reserpin und einigen Monoaminen weiter abgeklärt werden. Dazu wurde der Einfluss von Iproniazid und Isoniazid auf die Temperaturwirkung von Reserpin und verschiedenen Monoaminen bei Kaninchen untersucht und mit LSD sowie Chlorpromazin verglichen. Chlorpromazin setzt im Gegensatz zu Reserpin kein 5HT frei⁵.

Methodik. Bei Kaninchen wurde 24 h nach Iproniazid-Behandlung bzw. ohne Vorbehandlung Reserpin, 5HT, Adrenalin, Tyramin, Phenyläthylamin, Mezcalin und Chlorpromazin injiziert. Weitere Tiere erhielten 24 h nach Isoniazidinjektion Reserpin und 5HT, sowie LSD ohne Vorbehandlung. Die rektale Temperatur wurde mit Thermoelementen zweimal vor und achtmal nach Verabreichung von Reserpin, Chlorpromazin und der Amine halbstündlich gemessen⁶.

Resultate.

a) 0,5 und 1 mg/kg Reserpin intravenös erzeugten bei Kaninchen Hypothermie, während LSD hypertherm

¹ B. B. BRODIE, A. PLETSCHER und P. A. SHORE, J. Pharmacol. exp. Therap. **119**, 9 (1956). – H. BESENDORF und A. PLETSCHER, Helv. physiol. pharmacol. Acta **14**, 383 (1956).

² A. PLETSCHER, P. A. SHORE und B. B. BRODIE, J. Pharmacol. exp. Therap. **116**, 84 (1956); Science **122**, 374 (1955). – P. A. SHORE, A. PLETSCHER, A. TOMICH, R. KUNTZMAN und B. B. BRODIE, J. Pharmacol. exp. Therap. **117**, 232 (1956). – A. CARLSSON und N. A. HILLARP, Kungl. Fysiografiska Sällskapets i Lund Förhandlingar **26**, Nr. 8 (1956). – M. BURGER, Helv. physiol. pharmacol. Acta **14**, C13 (1956). – M. HOLZBAUER und M. VOGT, J. Neurochem. **1**, 8 (1956).

³ A. SJOERDSMA, T. E. SMITH, T. D. STEVENSON und S. UDENFRIEND, Proc. Soc. exp. Biol. Med. **89**, 36 (1955). – E. A. ZELLER, J. BARSKY, J. R. FOUTS, W. F. KIRCHHEIMER und L. S. VAN ORDEN, Exper. **8**, 349 (1952). – E. A. ZELLER, J. BARSKY, E. R. BERMAN und J. R. FOUTS, J. lab. Clin. Med. **40**, 965 (1952). – E. A. ZELLER und J. BARSKY, Proc. Soc. exp. Biol. Med. **81**, 459 (1952). – R. W. SCHAYER, Proc. Soc. exp. Biol. Med. **84**, 60 (1953). – E. A. ZELLER, J. BARSKY und E. R. BERMAN, J. biol. Chem. **214**, 267 (1955).

⁴ B. B. BRODIE, A. PLETSCHER und P. A. SHORE, J. Pharmacol. exp. Therap. **119**, 9 (1956). – B. B. BRODIE und P. A. SHORE, Ann. N. Y. Acad. Sci. (1956), im Druck. – H. BESENDORF und A. PLETSCHER, Helv. physiol. pharmacol. Acta, **14**, 383 (1956).

⁵ B. B. BRODIE, P. A. SHORE und A. PLETSCHER, Science **123**, 992 (1956).

⁶ Die Temperaturmessung wurde im pharmakologischen Laboratorium der F. Hoffmann-La Roche & Co. A.-G. (Leiter: Dr. B. PELLMONT) durchgeführt.

Präparat	Dosis mg/kg		Mittlere Beeinflussung der Körpertemperatur in °C (je drei Tiere)	p = *	p = **
Ohne Vorbehandlung					
Reserpin	0,5	intravenös	– 0,92	0,04	
Reserpin	1,0	intravenös	– 1,33	0,001	
5-Hydroxytryptamin	25,0	intravenös	+ 0,34	0,05	
Adrenalin	0,25	subkutan	– 0,07***	0,7	
Tyramin	37,5	intravenös	+ 0,57	0,001	
Phenyläthylamin	8,5	intravenös	+ 0,34	< 0,001	
Mezcalin	50,0	subkutan	+ 0,34	0,008	
LSD	0,02	subkutan	+ 1,20	< 0,001	
	0,05	subkutan			
Chlorpromazin	5,0	intravenös	+ 1,39	< 0,001	
			– 0,61	0,001	
Vorbehandlung mit 100 mg/kg Iproniazid intravenös					
Reserpin	0,5	intravenös	+ 1,09	< 0,001	< 0,001
Reserpin	0,75	intravenös	+ 1,83	< 0,001	
5-Hydroxytryptamin	25,0	intravenös	+ 1,83	< 0,001	< 0,001
Adrenalin	0,25	subkutan	+ 0,77***	0,05	0,002
Tyramin	37,5	intravenös	+ 2,87	< 0,001	< 0,001
Phenyläthylamin	8,5	intravenös	+ 1,64	0,01	< 0,001
Mezcalin	50,0	subkutan	+ 2,28	0,001	< 0,001
Chlorpromazin	5,0	intravenös	– 1,03	< 0,001	0,5
Vorbehandlung mit 100 mg/kg Isoniazid intravenös					
Reserpin	1,0	intravenös	– 1,04	< 0,001	0,45
5-Hydroxytryptamin	25,0	intravenös	– 0,14	0,5	

* Vergleich mit Ausgangswerten. ** Vergleich mit entsprechender Gruppe ohne Vorbehandlung. *** 2-Stunden-Versuch.

- wirkte (siehe auch ⁷). Nach Iproniazidvorbehandlung verursachten 0,5 und 0,75 mg/kg Reserpin intravenös Erhöhung der Körpertemperatur, 1 mg/kg Reserpin intravenös bewirkte letale Hyperpyrexie.
- b) Bei nicht mit Iproniazid vorbehandelten Tieren hatten 5HT, Tyramin, Phenyläthylamin und Mezcalin leicht hyperthermen, 0,25 mg/kg Adrenalin keinen Effekt. Nach Vorbehandlung mit Iproniazid wurde die hypertherme Wirkung signifikant verstärkt, und auch 0,25 mg/kg Adrenalin verursachten signifikante Temperatursteigerung.
- c) Isoniazidvorbehandlung beeinflusste weder den Temperatureffekt von Reserpin, noch von 5HT.
- d) Die hypotherme Wirkung von Chlorpromazin wurde durch Iproniazidvorbehandlung nicht abgeschwächt.

Diskussion. Nach den erwähnten Befunden erzeugte Reserpin bei Kaninchen nach Iproniazidvorbehandlung nicht nur Exzitation, Mydriasis und Pilo-Erektion, sondern auch Steigerung der Körpertemperatur. Die Kombination Iproniazid/Reserpin hatte also auch in bezug auf Beeinflussung der Körpertemperatur LSD-artige Wirkung.

Bei der Erhöhung der Körpertemperatur durch Reserpin und 5HT nach Iproniazidbehandlung spielte

wahrscheinlich Hemmung der Monoaminoxidase eine Rolle. Dies geht daraus hervor, dass Isoniazid, welches die Monoaminoxidase nicht wesentlich hemmt, die Temperaturwirkung von Reserpin und 5HT nicht beeinflusste.

Die Befunde über Beeinflussung des Temperatureffektes von Reserpin, 5HT, Adrenalin und Chlorpromazin durch Iproniazid sprechen dafür, dass die Temperaturerhöhung nach Iproniazid/Reserpinbehandlung durch freigesetzte endogene Monoamine, zum Beispiel 5HT, Adrenalin, zustande kam. Reserpin, das Freisetzung von Monoaminen aus dem Gehirn bewirkt, hatte beim nicht-iproniazidbehandelten Tier temperatursenkende Wirkung. Nach Iproniazidvorbehandlung erzeugte es Hyperpyrexie. Dies kann durch erhöhte Konzentration von freigesetzten endogenen Aminen im Gehirn infolge Monoaminoxidasehemmung erklärt werden. Auch 5HT und Adrenalin hatten nach Iproniazidvorbehandlung deutliche Temperatursteigerung zur Folge. Die hypotherme Wirkung von Chlorpromazin wurde durch Iproniazidbehandlung nicht abgeschwächt. Chlorpromazin bewirkt keine 5HT-Freisetzung, eine Akkumulation von freiem 5HT im Gehirn ist also nicht möglich.

Exzitation und Hyperpyrexie nach Iproniazid/Reserpinbehandlung sind nicht bei allen Tierarten ausgeprägt. Bei Mäusen bewirkte Iproniazidvorbehandlung lediglich Abschwächung der sedativen und hypothermen Wirkung von Reserpin⁸.

⁷ H. J. BEIN, *Exper.* 9, 107 (1953). – H. J. BEIN, J. TRIPOD und R. MEIER, *Schweiz. med. Wschr.* 83, 1007 (1953). – A. HORITA und J. M. DILLE, *Science* 120, 1100 (1954). – Z. SUPEK und S. MILKOVIC, *Lancet* 1955/II, 562.

⁸ A. W. LESSIN und M. W. PARKES (1957), i.d. Vorbereitung.

Nach Iproniazid-Applikation hatten nicht nur 5HT und Adrenalin, sondern auch andere Monoamine (Phenyläthylamin, Tyramin, Mezcalin) verstärkte temperatursteigernde Wirkung. Durch Hemmung der Monoaminoxidase fällt möglicherweise ein allgemeiner Schutzmechanismus des ZNS gegen die Einwirkung von Monoaminen aus. Die Befunde bei kombinierter Iproniazid/Reserpin-Applikation lassen daran denken, dass endogene Monoamine (zum Beispiel 5HT, Adrenalin, Noradrenalin) bei gestörter Aktivität der Monoaminoxidase erregende Wirkung auf das ZNS ausüben können.

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Medizinische Forschungsabteilung der F. Hoffmann-La Roche & Co., AG., Basel, den 31. Januar 1957.

Summary

The influence of isopropyl-isonicotinic acid hydrazide (Iproniazid) on the course of body temperature after reserpine, 5HT, adrenaline, various other monoamines, as well as chlorpromazine, was investigated in rabbits and compared with the temperature effect of lysergic acid diethylamide. Furthermore, the influence of isonicotinic acid hydrazide (Isoniazid) on the temperature effect of reserpine and 5-hydroxytryptamine (5HT) was measured. The following results were obtained:

- 1) After pretreatment with Iproniazid, reserpine no longer produced hypothermia, but marked hyperthermia.
- 2) The hyperthermic effect of 5HT and various other monoamines was significantly increased by Iproniazid-pretreatment.
- 3) Isoniazid-pretreatment did not influence the temperature effect of reserpine and 5HT.
- 4) The hypothermic effect of chlorpromazine was not significantly changed by Iproniazid-pretreatment.

DISPUTANDUM

Effects of Pyrithiamine and Oxythiamine on the Thiamine Content of Tissues and Blood Pyruvate in Mice

DE CARO *et al.* have stated in their paper in *Experientia* 12, 300 (1956) that WOOLLEY and MERRIFIELD based their evidence on the fact that pyrithiamine did not decrease the thiamine content of tissues. This statement is not true. WOOLLEY and MERRIFIELD showed that the cocarboxylase content of tissues and the pyruvate content of blood were not reduced by pyrithiamine. Actually they feel very strongly that pyrithiamine should reduce the thiamine content of tissues. The fundamental mode of action of an antimetabolite is to displace the related metabolite and thus to lower the content of this metabolite in the tissues. In the case of pyrithiamine then, one would expect the thiamine content of the tissues to be lowered.

The whole point of WOOLLEY and MERRIFIELD's argument was not that the thiamine content was unchanged, but rather that the cocarboxylase content was not changed. Consequently, the demonstration by DE CARO

et al. that the thiamine content of brain is decreased in animals previously treated with pyrithiamine does not differ from the concept of WOOLLEY and MERRIFIELD and most certainly cannot be taken as evidence that the view of the latter authors with respect to a new function for thiamine is erroneous.

D. W. WOOLLEY

We think that in our paper the essential points of WOOLLEY and MERRIFIELD hypothesis are clearly exposed, the importance of cocarboxylase content of the tissues being sufficiently emphasized.

In fact exposing our experimental data we clearly referred to the total thiamine content, once in the text and once in the table. The term 'total thiamine' cannot be misleading: as is well known, it means free thiamine + phosphorylated thiamine (cocarboxylase). After the indications in the text and in the table, we spoke often of thiamine, but, obviously, always in the sense of total thiamine: we did not consider it necessary to repeat it every time.

Further, it is well known that the thiamine in the tissues is nearly all in the form of cocarboxylase, especially in the nervous and muscular tissues.

In particular in normal mice brain, we recently found the following results:

total thiamine μg 3.16/g
free thiamine μg 0.05/g

Hence it is clear that a significant decrease of the total thiamine can only be a consequence of a significant decrease of the cocarboxylase. Now, it is out the question that the Pyrithiamine sharply lowers (from 2.16 to 0.74 $\mu\text{g/g}$) the total thiamine content and, therefore, the cocarboxylase content in the mice brain.

In our recent paper [*Intern. Z. Vitaminforsch.* 26, 343 (1956)] on the rats (treated by mg 2.5 of Pyrithiamine for 6 days), we were able to show the behaviour of free and phosphorylated thiamine (or cocarboxylase). Actually the Pyrithiamine lowered, in these experiments, the brain phosphorylated thiamine from 2.8 $\mu\text{g/g}$ to 0.72 $\mu\text{g/g}$, while the free thiamine decreased from 0.08 $\mu\text{g/g}$ to 0.02 $\mu\text{g/g}$. In the muscle and in the liver too, the Pyrithiamine lowered the phosphorylated thiamine content.

Therefore we still consider our conclusion right that the WOOLLEY and MERRIFIELD hypothesis, based on the claim that the Pyrithiamine does not modify the cocarboxylase content of the tissues, is not experimentally sustainable.

L. DE CARO, G. RINDI, V. PERRI,
and G. FERRARI

Allow me to say once again that DE CARO *et al.* have not stated the findings of WOOLLEY and MERRIFIELD correctly. Their findings about thiamine content of tissues are *not* contradictory to the observations of WOOLLEY and MERRIFIELD and consequently they are unjustified in stating in your journal that they have been unable to confirm the experimental findings on which WOOLLEY and MERRIFIELD based their conclusions. WOOLLEY and MERRIFIELD made their measurements of cocarboxylase. DE CARO, RINDI, PERRI, and FERRARI are consequently unjustified in stating in their paper that WOOLLEY and MERRIFIELD made measurements on thiamine. Thiamine is not the same substance as cocarboxylase.

Although it is widely believed that much of the thiamine in normal tissues is present in a combined form, it

is by no means proven that this is the case in animals treated with pyriithiamine and in fact it is not accepted by all investigators that all of the combined thiamine is identical with cocarboxylase. Consequently, the reply of DE CARO, RINDI, PERRI, and FERRARI to my previous objection is not convincing.

D. W. WOOLLEY

With this I declare: End of the discussion. The Redactor.

PRO EXPERIMENTIS

Detection of Dipeptides and Dipeptidase Activity on Paper

During the course of some work¹ on the purification of pig kidney cysteinyl-glycinase², the usefulness of working out a quick method to detect the dipeptidase activity became apparent.

The method is a modification of GIRI and NAGABHUSHANAN's test for the detection of amino acids on paper³, and is based on the observation that, after spraying with naphtoquinonesulfonate and during the following alcohol-alkali treatment, some dipeptides change their colour at a lower rate or develop a different colour than the corresponding amino acids, thus enabling the use of this reaction for the detection of dipeptidase activity.

Procedure. Reagent *a*: 0.3 g of Na β -naphtoquinone-4-sulfonate (Eastman) is dissolved in 10 ml water, and distilled acetone is added to 100 ml. The reagent must be prepared immediately before use.

Reagent *b*: 2 ml of 4 *N* NaOH is diluted to 100 ml with 95% ethanol.

The paper is sprayed with reagent *a*, and heated in an oven at 100°C for 3–5 min. At this stage, amino acids and dipeptides develop colours which are often different enough to permit one to distinguish between them: cysteinyl-glycine, e.g., gives a pink-yellow colour; glycine and cysteine a violet one.

A better differentiation is obtained if the paper is then dipped in a bath of reagent *b*. The colours of most of the amino acids turn to grey-green within 10–15 min, whilst most of the dipeptides tested retain for a longer time the staining acquired during the heating, or develop a different colour. After some hours' bath, the contrast is much less clear.

Colours of some dipeptides and of the corresponding amino acids after 10 min treatment with alcohol-alkali.

Glycyl-glycine	–violet-brown	–Cysteine	– bluish-green
Glycyl-leucine	–violet-brown	–Glycine	– bluish-green
Glycyl-tyrosine	–violet-brown	–Leucine	– bluish-green
Glycyl-tryptophan	–violet-brown	–Alanine	– bluish-green
Leucyl-glycine	–green-yellow	–Tyrosine	– grey-brown
Alanyl-glycine	–green-yellow	–Tryptophan	– grey-brown
Cysteinyl-glycine	–pink-yellow		
(+ glutamic acid)			

Instead of cysteinyl-glycine, a mixture of cysteinyl-glycine and glutamic acid (partial acidic hydrolysate of glutathion⁴) was used. All substances were Hoffmann-La Roche products.

¹ G. SEMENZA, *Biochim. biophys. Acta* 1957 (in press).

² F. BINKLEY, *Exp. Cell. Res. Suppl.* 2, 145 (1952).

³ K. V. GIRI and A. NAGABHUSHANAN, *Naturwissenschaften* 39, 548 (1952).

As seen from the above table, a differentiation is possible between several dipeptides and their respective component amino acids.

Test for the cysteinyl-glycinase activity. The following procedure was used either to detect the cysteinyl-glycinase on the electrophoresis paper, or as a spot test of a solution to be analyzed.

The filter paper carrying a drop of the solution, or the electrophoresis paper, was dipped in a uniform thin layer of the substrate-buffer-activator mixture: neutralized, hydrolyzed glutathion⁴ 3 to 4 mg per millilitre, corresponding to about 1.5 to 2 mg cysteinyl-glycine per millilitre, in 0.02 *M* THAM-HCl⁵ buffer, pH 8.1 + 0.0005 *M* MnCl₂.



Paper electrophoresis of cysteinyl-glycinase. 0.04 *M* THAM-HCl buffer, pH 8.2, + 0.0005 *M* MnCl₂; 400 V during 3 h at room temperature on Whatman N 54. The cysteinyl-glycinase activity is shown by the green-grey spot (white in the figure) against a pink background (black in the figure).

Incubation was carried out at room temperature for 30–45 min, and stopped by heating the paper in the oven at 110°C for 5 min. The paper was then stained according to the procedure described above; the cysteinyl-glycinase activity was shown by the appearance of a violet (after the heating) or green-bluish (in the alcohol-alkali bath) spot, against a pink-yellow background.

Alternatively, the incubation was carried out by superposing the paper into a 1% agar gel, containing the substrate-buffer-activator mixture. The agar gel was then stained by spraying the reagent *a*: the colour developed slowly at room temperature, without any further treatment. The cysteinyl-glycinase activity was shown by the appearance of a brown spot, against a yellow background. The electrophoresis paper, in this alternative procedure, could of course be used for other analysis.

The sensitivity of these procedures is somewhat higher than the usual quantitative method in a tube⁴.

To test other dipeptidase activities, an identical procedure was used. The reaction mixture contained about 3 mg of dipeptide per millilitre, the buffer and the suitable activator.

G. SEMENZA*

Institute of Biochemistry, University of Uppsala, Sweden, December 19, 1956.

Riassunto

Viene descritto un metodo rapido e sensibile per la rilevazione delle dipeptidasi su carta.

⁴ F. BINKLEY, *J. biol. Chem.* 186, 731 (1950).

⁵ Tris-hydroxymethyl-amino-methane Sigma.

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PRO EXPERIMENTIS

Mechanism of Elastolysis by Pancreatic Elastase

Acetate extracts of pancreas powder contain an enzyme system which dissolves elastin from aorta media, tendon and also heat-contracted collagen¹. In order to study the mechanism of elastolytic activity, we worked out a simple method based on the spectrophotometric measurement of the rate of dissolution of 'azoelastin', prepared by the method used by OAKLEY *et al.*² for the preparation of azocollagen. This comprises coupling elastin (from aorta media, BANGA³) with diazobenzidine-naphtholdisulphonic acid (details of the method will be given in another paper).

'Azoelastin' as well as elastin is only very slowly digested by crystalline trypsin⁴: at pH 9.5–9.7 (glycine-NaOH-buffer, 0.1 M)⁵ 2 mg azoelastin is dissolved in 24 h by 0.88 mg trypsin. Crystalline chymotrypsin⁴ works somewhat better: 1.2 mg of this enzyme dissolved 17 mg azoelastin in 2 h and 74 mg in 7 h at 35°C, in the same conditions as for trypsin. The mixture of these two enzymes dissolved the same quantity. Crystalline pepsin⁴ dissolved azoelastin completely in a few hours at pH 1–2. The proteolytic factor (now called elastase by BANGA⁶) of one of our elastase preparations (obtained by the Banga-Balo method³) had a proteolytic activity equivalent to 229 γ of crystalline trypsin per milligram total protein, as determined by NORTHROP's hemoglobin-method⁷. This activity is somewhat lower than that found by PARTRIDGE and DAVIS⁸.

The same preparation dissolved 18.6 mg azoelastin per milligram enzyme protein in 30 min at 35°C and about 10 mg of this enzyme gave complete dissolution of 200 mg azoelastin in 2 h.

The elastin-elastase system is suitable for the study of the heterogenous enzyme kinetics. The first step of the enzymatic dissolution process seems to be the rapid adsorption of enzyme onto the insoluble substrate. This is demonstrated by the following experiment: different quantities of elastase are added to the same quantity (200 mg) of azoelastin in 3 ml acetate buffer 0.1 M pH 4.9 with water added to 5 ml. After 30 min shaking at 20°C the azoelastin is centrifuged down and washed out twice with 3 ml acetate buffer, siphoned and kept overnight at 0°C. After heating to 35°C 4 ml glycine-NaOH buffer pH 9.7, 0.1 M is added with 2 ml water and elastolysis is followed spectrophotometrically at 520 m μ . The first figure gives some typical curves of this experiment together with control curves. It is clear that preincubation with the adsorbed enzyme resulted in a modification of the substrate, the lag period of the digestion disappears. The second figure gives the slopes of the linear portions of the curves as a function of total initial enzyme concentration, for the adsorption experiment

and the control series. At low enzyme concentrations dissolution is faster in the control series, but for higher

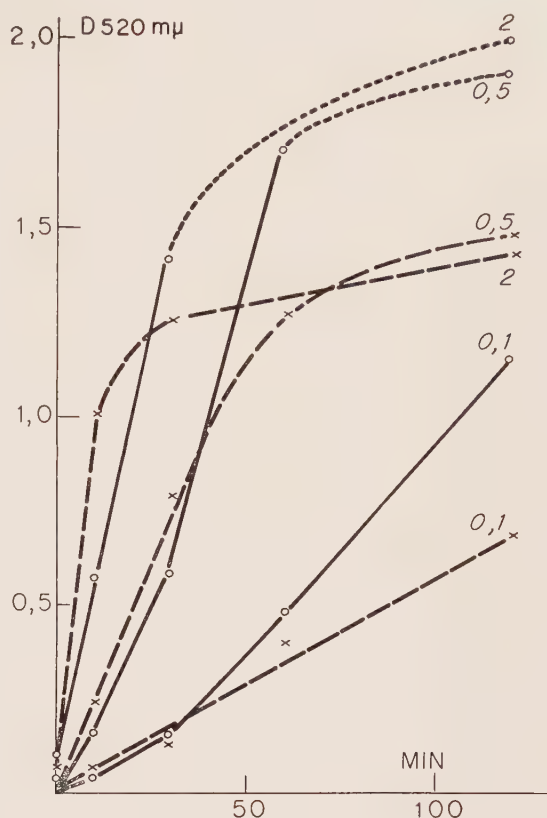


Fig. 1. — Kinetics of dissolution of azoelastin by preadsorbed enzyme (---) and freshly added enzyme (—). Ordinates: optical density at 520 m μ . The figures on the curves indicate the quantity of enzyme in ml added to 200 mg azoelastin. In the adsorption experiments non adsorbed enzyme was removed.

enzyme concentrations adsorbed enzyme acts much more rapidly. This could be interpreted as a preliminary

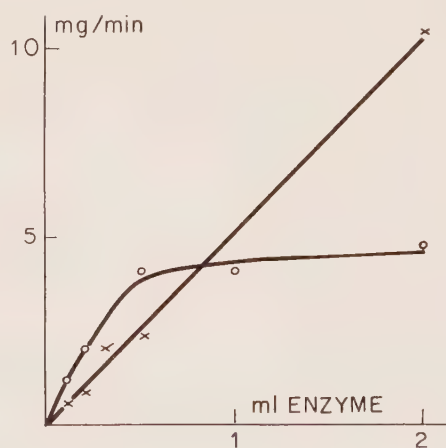


Fig. 2. — Abscissa: milliliters of enzyme added to 200 mg azoelastin. In adsorption-experiments the non-adsorbed enzyme was removed. Ordinate: speed of elastolysis in mg azoelastin dissolved per minute at 35°C, pH 9.6, $\circ$ — $\circ$ freshly added enzyme, $\times$ — $\times$ preadsorbed enzyme.

¹ J. BALO and I. BANGA, *Biochem. J.* **46**, 384 (1950). — I. BANGA, *Nature* **172**, 1099 (1953).

² C. L. OAKLEY, G. HARRIET, and W. E. VON HEYNINGEN, *J. Path. Bact.* **58**, 229 (1946).

³ I. BANGA, *Acta physiol. Acad. Sci. Hung.* **3**, 317 (1952).

⁴ We thank Worthington Biochemicals, New Jersey, for the generous gift of crystalline trypsin, chymotrypsin and pepsin.

⁵ J. BALO and I. BANGA, *Biochem. J.* **46**, 384 (1950).

⁶ I. BANGA and J. BALO, *Nature* **178**, 310 (1956).

⁷ J. H. NORTHROP, M. KUNITZ, and R. M. HERRIOTT, *Crystalline Enzymes* (Columbia Univ. Press 1948), p. 307.

⁸ S. M. PARTRIDGE and H. F. DAVIS, *Biochem. J.* **61**, 21 (1955).

modification of azoelastin during the incubation by a slow enzymatic process of low activation energy rendering

accessible the substrate to the proteolytic component. It is not impossible that this preliminary reaction, taking place during the lag phase, could be the mucolytic process mentioned by BANGA and BALO⁶. We could verify that the adsorption mechanism holds for the dissolution of non-diazotised aorta-elastin too.

L. ROBERT and P. SAMUEL*

Service of biological Chemistry, Faculty of Medicine, Paris, December 4, 1956.

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Résumé

Nous présentons une étude du mécanisme de l'élastolyse par une élastase partiellement purifiée du pancréas. La méthode utilisée consiste dans le dosage spectrophotométrique de l'«azoeastine» dissoute. Cette protéine n'est que très lentement hydrolysée par la trypsine et chymotrypsine cristallisées. L'élastase possède une activité protéasique considérable. L'enzyme est très rapidement adsorbée sur l'élastine. Après une incubation à pH 4,5 avec l'enzyme adsorbée, le temps de latence de l'élastolyse disparaît et la rapidité de la réaction devient proportionnelle à la concentration en élastase.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

The Metabolism of the Proteins of the Brain*

By A. LAJTHA, S. FURST**, and H. WAELSCH

Of the major components of the brain, least attention has been paid to the protein constituents. The large concentration of lipids and the process of myelination, unique for the nervous tissue and so easily visualized by histological techniques, have for many years attracted the interest of biochemists. With the development of our understanding of the role of carbohydrates in the energy metabolism of living tissue, the last 25 years witnessed an ever increasing concern with this aspect of intermediary metabolism of the brain. Only recently have the amino acids and proteins of the central nervous system been subjected to more intensive study, but these investigations have been and are essentially analytical in nature. Hardly any other organ system offers as many fascinating and far reaching implications for the function of its component proteins as does the nervous system.

This laboratory has for many years been interested in the metabolism of the amino acids and proteins of the nervous system, and we should like to summarize in this report our studies dealing with the turnover of the proteins of the whole brain, of different parts of the brain, and of different cell fractions¹. Incidental to these investigations, studies of the penetration of lysine into the brain of young and adult animals as well as observations on protein metabolism in the immature brain have been made.

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** Special Fellow of the U. S. Public Health Service.

¹ The work described in this report was supported by grants (B-226 and 557) of the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, by a contract between the Office of Naval Research and the Psychiatric Institute and by a grant from the Supreme Council, 33th Scottish Rite Masons of the Northern Jurisdiction, United States of America. Helpful discussions with Drs. D. RITTENBERG and J. REINER are gratefully acknowledged. A preliminary report of this work was presented. A. LAJTHA and S. FURST, in *Symposium on Neurometabolism* (Paul Hoeber, Inc., New York 1955) (in press).

The study of the turnover of brain proteins by the determination of the rate of uptake of an isotopically labelled amino acid is made difficult by the existence of the blood-brain barrier which decreases the rate of transfer of many metabolites from the circulating blood to the brain. Some compounds are, for all practical purposes, excluded from the brain². For example, no increase of the glutamic acid concentration in brain could be detected after a 30 fold increase of this amino acid in the blood. On the other hand, a significant, although small, increase of the glutamine concentration in brain was found after intravenous administration of the amide to rats or mice³. It is not surprising that isolated observations of lack of incorporation of amino acids, parenterally administered, led to the conclusion that the brain proteins have a very slow turnover⁴. When, on the other hand, amino acids were introduced directly into the subarachnoidal spaces, in order to avoid the blood-brain barrier, considerable incorporation into proteins was observed⁵.

The design of the experiments.—For reasons to be discussed below, it appeared desirable to select the physiological path of supply of amino acids to the brain, i.e., from the circulating blood. Lysine was chosen as the test amino acid, there being reason to believe that this basic amino acid would penetrate the blood-brain barrier at a faster rate than an acidic or neutral one⁶. The technique followed, with minor variations, in the experiments to be reported, was as follows:

Lysine C¹⁴ was injected intravenously or intraperitoneally into mice and monkeys, and the brains, livers and other organs were removed after definite time intervals. The protein fractions obtained by precipitation with trichloroacetic acid and extraction with organic

² H. WAELSCH in *Biochemistry of the Developing Nervous System* (H. WAELSCH ed., Academic Press, Inc., New York 1955).

³ P. SCHWERIN, S. P. BESSMAN, and H. WAELSCH, *J. biol. Chem.* **184**, 37 (1950).

⁴ H. BORSOOK and C. L. DEASY, *Ann. Rev. Biochem.* **20**, 209 (1951).

⁵ F. FRIEDBERG, H. TARVER, and D. GREENBERG, *J. biol. Chem.* **173**, 355 (1948). — M. K. GAITONDE and D. RICHTER, *Biochem. J.* **59**, 690 (1955).

⁶ H. WAELSCH in *Biochemistry of the Developing Nervous System* (H. WAELSCH ed., Academic Press, Inc., New York 1955). — P. SCHWERIN, S. P. BESSMAN, and H. WAELSCH, *J. biol. Chem.* **184**, 37 (1950).

solvents were measured for their radioactivity, and, after hydrolysis, the lysine content was determined by microbiological assay for calculation of specific activities. Similarly, in the nonprotein fraction of each organ, radioactivity and lysine content were determined. In control experiments it was ascertained that during the experimental period less than 1% of the radioactivity administered as lysine was found in amino acids other than lysine. Postmortem changes owing to manipulation of the excised organs did not influence the results.

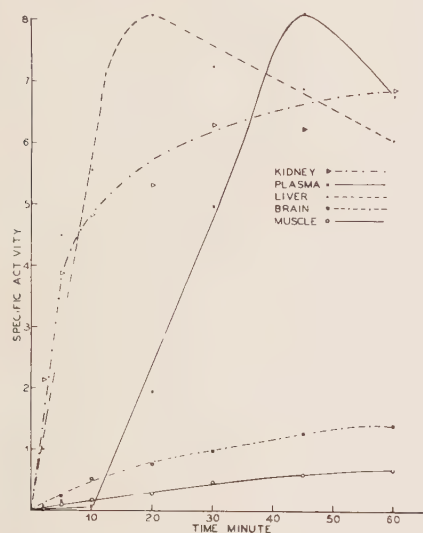


Fig. 1. — Specific activity of protein bound lysine of various organs (counts/γ lysine/min) after intravenous injection of lysine C¹⁴ in adult mice.

The results of a typical experiment in which groups of mice were sacrificed at different time intervals after intravenous injection of radioactive lysine are shown in Figure 1. It will be noted that the experimental periods chosen are in minutes. This is in contrast to experiments employing considerably longer time intervals in which the half life time of proteins was calculated from the decay of radioactivity or N¹⁵ content of a protein after administration of a labelled amino acid⁴. These latter experiments supply data for calculation of the turnover of the bulk of the slowly metabolized proteins of an organ. They do not, however, offer information on rapidly metabolized proteins from which the labelled amino acid has disappeared in the experimental period and has been diluted out by the pool of non-labelled free amino acids. On the other hand, experiments of the type described in the present communication must be cautiously interpreted because of the rapid changes occurring during the short-time interval. The results of the experiment shown in Figure 1 demonstrate that within minutes after administration, labelled lysine appears in the brain proteins despite the fact that the amino acid had been administered without circumvention of the blood-brain barrier.

The renewal of the amino acid pool as measured with isotopic lysine.—According to the prevailing views proteins draw their constituent amino acids from the free amino acids of the nonprotein fraction. The calculation of turnover rates of the proteins of an organ requires knowledge of the specific activity of the test amino acid in the amino acid pool of the organ⁷. In our experiments,

Table I. — Flux (F) of free lysine into mouse brain and liver
γ lysine/g fresh tissue/min

Duration of experiment minutes	Brain				Liver		
	Exp. III	Exp. V	Exp. VII	Exp. VIII	Exp. III	Exp. VII	Exp. VIII
	Adult	Adult	Young	Young	Adult	Young	Young
2	2.3				29		
3		0.7					
5		1.3	1.2	1.1	10		
10			1.5	1.1	20	24	63
20	5.2				6.9	8.5	
30	1.5				6.3		
45						11	8.4

Adult mice: 120 days old; young mice: 10 days old.

$F = C (dSb^{***}/dt) / Sa - Sb$ where C = γ free lysine in g fresh tissue; Sa = specific activity of plasma free lysine; Sb = specific activity of organ free lysine.

*** Estimated graphically

considerable radioactivity was found in the free amino acid fraction of the brain of mice even when the animals were killed 2 min after the administration of the isotopic amino acid (Fig. 2). This observation suggests that the blood-brain barrier is permeable to lysine. The transfer coefficient or flux of free lysine from blood to the brain of mice may be estimated on the basis of the concentrations of isotopic lysine found in the plasma and in the nonprotein fraction of the organ (Table I). These estimates can only be approximate since they are based on the rapidly changing isotope concentration in the plasma during the first minutes of the experiment. The values are expressed in γ of lysine transferred per minute from blood to brain and show a considerable range varying from 0.7 to 5.2 γ/g tissue/min. From these values the time interval in which one half of the free lysine was replaced was calculated. It was found not to exceed 45 min for both young and adult mice. The same calculation when applied to replacement of half the free lysine of liver leads to values not greater than 10 min.

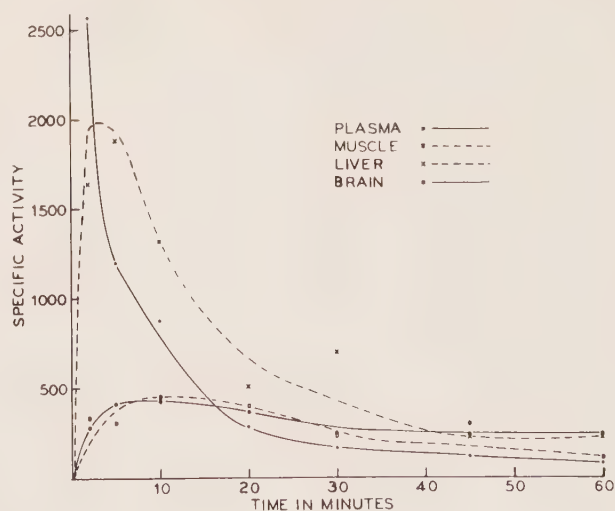


Fig. 2. — Specific activity of free lysine of various organs (counts/γ lysine/min) after intravenous injection of lysine C¹⁴ in adult mice.

In addition to exchange, or turnover rate, the problem of net uptake presents itself. Accordingly, it was attempted to increase the concentration of the free lysine

⁷ O. B. HENRIQUES, S. B. HENRIQUES, and A. NEUBERGER, Biochem. J. 60, 409 (1955).

Table II.—Half-life time (days) of proteins of mouse organs

Time After injection in minutes	Brain		Liver		Muscle	
	Adult	Young	Adult	Young	Adult	Young
2	2.8		0.90		3.5	
5	3.5	3.4	1.25	1.73	7.6	2.4
10	5.5	2.2	2.57	1.10	7.6	1.7
20	6.2	3.0		2.68	10.4	2.5
30	6.9				13.2	
45	10.4	2.4			16.6	2.3
60	15.2				23.6	

Half-life time was calculated according to the following equation⁸:

$$\frac{1}{2} \text{ life in days} = C \times \ln 2 / F \times 1440$$

$C = \gamma$ lysine/mg dry protein; $F =$ flux of lysine (γ lysine incorporated/mg protein/min).

of the brains of young (10 days old) and adult mice by intravenous injection of large amounts of nonisotopic amino acid. By raising the concentration of the free lysine of the plasma 25–50 fold, increased brain concentrations were effected. These averaged 100% in the young animal, while the significance of the changes in lysine concentration of the brain of adult animals is questionable.

Thus while the experimental evidence is not conclusive, it suggests that in brains of both young and adult mice the free lysine is *renewed* at comparable rates although larger *net uptake* of lysine can be induced in the brain of the former.

The half-life time of the brain proteins.—The flux of lysine from the nonprotein nitrogen fraction into the proteins was calculated on the basis of the specific activity of the free lysine and the protein-bound lysine of the brain and other organs at given times. These data are expressed as half-life time in order to facilitate comparison with values recorded in the literature (Table II). In the experiments with adult mice the calculated half-life time of the protein increased with each increasing time interval between administration of the amino acid and the sacrifice of the animal. The same relationship was found for the half-life time of muscle and liver protein. It should be pointed out that all derivations developed for calculation of half-life of turnover time of proteins rest on the assumption of a homogeneous system in which the component proteins have similar turnover rates. Such a situation may be simulated when the turnover of protein is determined from decay curves since here the bulk of the proteins with a slow turnover rate are measured, and these may appear as a homogeneous system. In experiments of short duration, the ascending portion of the activity time-curve is measured and in these, the various rapidly metabolized protein fractions will be prominent. The spectrum of turnover values obtained in our experiments reflects the metabolic inhomogeneity of the protein of the organs investigated, a fact which by itself hardly needs experimental verification.

When the same experiments were carried out with immature mice it was found that the fractions of slowly metabolized proteins were missing, while the turnover rates of the rapidly metabolized proteins were little altered (Table II).

⁸ D. B. ZILVERSCHMIT, C. ENTENMAN, and M. C. FISHLER, *J. gen. Physiol.* 26, 325 (1943). — J. M. REINER, *Arch. Biochem. Biophys.* 46, 53 (1953).

Table III.—Half-life times of proteins in monkey brain areas

Areas of brain	Half-life time in days calculated from different time points		
	5 min	10 min	45 min
Cord	10.6	9.5	18.5
White	3.9	4.0	8.7
Medulla-Pons	6.6	6.7	13.7
Thalamus-Hypothalamus	6.1	7.8	16.8
Cerebellum	5.4	7.6	14.1
Cerebral cortex	5.5	6.7	12.7

Further insight into the distribution of proteins with varying turnover rates may be obtained by the study of the metabolic activity of the proteins of the separate intracellular fractions, as well as of the metabolic picture characteristic of different parts of the organ. In an organ which is relatively homogeneous morphologically, such as the liver, the proteins of different turnover rates will be distributed throughout the organ. In an organ as morphologically inhomogeneous as the brain, the concentrations of proteins of different turnover rates may vary considerably from one area to another.

The turnover of proteins in different parts of the brain. In studying the turnover rate of proteins of different parts of the brain, adult Macaque monkeys (*Macaca Iru*) were used (Table III), since in these animals the ratio of brain to body weight is particularly high. Although care was taken in the preparation of the designated areas of the brain, inclusion of a certain amount of tissue from neighbouring areas could not be completely avoided. The highest rate of incorporation was found in the corpus callosum followed by the cortex. The spinal cord showed the lowest rate of incorporation. It is unquestionably striking and unexpected to find the highest rate of incorporation in the white matter of the corpus callosum and its radiation where fibres and oligodendroglia cells predominate. Without further study this finding should not be taken to mean that the turnover of the neuronal protein of the cortex is slower than that of the glial elements since no estimate of the contribution of neuronal or glial proteins to the over all picture can be made at the present time. Also it has been suggested that the site of synthesis of at least some of the axonal proteins is in the neuronal cell⁹ and it may therefore not be justified to relate the rate of incorporation of amino acids into the proteins of the white matter to the amino acid pool of this area. Regardless of the ultimate answer to questions of the distribution of proteins of different turnover rates between neuronal and non-neuronal elements the various areas of the brain contribute to the picture of inhomogeneity of turnover rates found in the analysis of the whole brain.

Turnover of the proteins of different cell fractions.—The cerebral cortex of Macaques, at various time intervals after the injection of isotopic lysine into the animals, was homogenized in isotonic sucrose solution and the homogenate fractionated by centrifugation¹⁰. The relative homogeneity of the fractions was confirmed by electron microscopy. Nevertheless, no definite statement as to the absolute purity of the individual fractions is attempted at this time. Of particular interest are those sediments which are obtained at high centrifugal forces—the microsomal fractions of cells. After removal of fractions at 800, 1500, and 12,000 $\times$ g, the remaining supernatant

⁹ P. WEISS and H. B. HISCOE, *J. exp. Zool.* 107, 315 (1948).

¹⁰ I. M. BRODY and J. A. BAIN, *J. biol. Chem.* 195, 685 (1952).

Table IV
Incorporation of lysine C^{14} into different cell fractions of brain cortex

Centrifugation		Counts/mg lysine/min.			Composition of Fraction
No. of gravities	Time in min.	Duration of Experiment minutes			
		5	15	30	
800	10	86	190	500	Cell debris, whole cells, nuclei
1500	10	140			Nuclei, large mitochondria
12000	15	270	200	394	Mitochondria
23000	30	390	670	790	Large microsomes
105000	30	540	860	1388	Small microsomes
105000	120			1346	Small microsomes
S		170	310	570	Supernatant fluid

fluid was subjected to succeeding centrifugations at 3,000 and 105,000 $\times g$ and the pellets collected. The specific activity of the lysine of the protein of these particulate fractions, as well as that of the protein remaining in the supernatant fluid, was determined (Table IV). In agreement with the results obtained with liver microsomes by other investigators¹¹, the microsomal fractions of brain incorporated lysine at a higher rate than other cell fractions. It should also be noted that the particles of the microsomal fractions obtained by centrifugation at higher speeds and for an extended period of time showed a higher specific activity than the microsomal fraction collected first. This finding agrees well with the observation that the smaller particles of the microsomal fraction of liver have a higher rate of incorporation than the larger microsomes in *in vitro* experiments¹². The proteins of the different cell fractions, therefore, add to the observed inhomogeneity of turnover rates of the brain proteins.

Discussion.—In approaching the problem of amino acid and protein metabolism of the central nervous system, the complexity of the organ presents considerable difficulties for the design of pertinent experiments. It should be recalled that the problem of the blood-brain barrier is only partially understood. While it is operative in the largest portion of the mature brain, there are areas in which its activity appears considerably diminished, such as the choroid plexus, the neural lobe and infundibulum of the pituitary, the pineal body, the area postrema, etc.¹³. Quantitatively, the absence of a blood-brain barrier in these structures may not seriously influence the uptake of substances when larger areas of the brain are studied. However, the cerebrospinal fluid originating in the choroid plexus undoubtedly must be considered because of the contribution it makes to the composition of the interstitial fluid through which any substance taken up by the cells has to pass. Substances introduced directly into the subarachnoid spaces circumvent the blood-brain barrier and follow the path taken by the cerebrospinal fluid. From the ventricles at least some will penetrate the adjacent parts of the brain and it cannot be assumed that their subsequent distribution in the brain will be the same as when offered from the

circulating blood. This is supported by the observation that the replacement of chloride in the cortex 3 h after the injection of bromide, iodide, or thiocyanate into the cisterna magna was found to be only one-fifth of that replaced in the cisternal fluid, while after intravenous injection, chloride replacement was the same in both cortex and cerebrospinal fluid¹⁴. Experiments in which radio phosphorus was introduced into the cerebrospinal fluid showed that the radioactivity was not distributed uniformly throughout the brain, but was greatest in the areas adjacent to the ventricles and decreased progressively in the more distant parts¹⁵.

Although lysine has a high exchange rate which permits the renewal of the free lysine of the brain within 1 h, the fully developed blood-brain barrier interferes with any extensive net uptake of the amino acid by the brain. This finding demonstrates that the blood-brain barrier also regulates the net uptake of small molecular basic substances, and again emphasizes its role in maintaining brain homeostasis. On the other hand, the immature brain permits a considerably greater net uptake of lysine from the circulating blood than does the mature brain, despite the fact that the exchange rates for the two are similar. This raises the question whether or not the same mechanisms govern both net uptake, and exchange of the amino acid. The fact that no net uptake of glutamic acid by the mature brain from the circulating blood could be demonstrated³ prompts the question as to whether the rate of exchange for acidic amino acids will be as high as for lysine. Differences in the exchanges rates of different amino acids would mean that the free amino acid pool of the brain is renewed at a specific rate for each amino acid or group of amino acids.

While half of the free lysine of the mouse brain is replaced in less than 45 min, the replacement of half of the potassium content of the rat brain may be calculated to require approximately 24 h¹⁶. It may be assumed that a similar relationship also holds true in the same animal. In view of the report that lysine can replace potassium in animals raised on a potassium-free diet¹⁷, the interesting possibility arises that the movements of the basic amino acids and of potassium may be interdependent.

In computing the turnover rates of the proteins of an organ, the specific activity of the labelled free amino acid in the organ must be taken into account. In addition to its high exchange rate between circulating blood and brain, lysine has the advantage over amino acids such as glycine⁷ or methionine¹⁸ in that it is converted only slowly to other amino acids, and therefore calculations of turnover rates based on its incorporation into proteins are simplified. Very recently experiments were reported in which methionine S^{32} was used for the study of protein turnover¹⁹. In these experiments labelled methionine was introduced into the cisterna magna in a concentration 100 fold that of the free methionine of the brain. By assuming the resulting activity of the methionine pool to be close to that of the administered methionine, the authors felt justified in omitting the determination

¹¹ E. B. KELLER, P. C. ZAMECNIK, and R. B. LOFTFIELD, J. Histochem. Cytochem. 2, 378 (1954).

¹² J. W. LITTLEFIELD, E. B. KELLER, J. GROSS, and P. C. ZAMECNIK, J. biol. Chem. 217, 111 (1955). — H. SACHS and H. WAELSCH, Biochim. biophys. Acta 21, 188 (1956).

¹³ E. W. DEMPSEY and G. B. WISLOCKI, J. biophys. biochem. cytology 1, 245 (1955).

¹⁴ J. B. WALLACE and B. B. BRODIE, J. Pharmacol. exp. Therap. 65, 220 (1939).

¹⁵ L. BAKAY, Arch. Neurol. Psychiat. 70, 30 (1953).

¹⁶ R. KATZMAN and P. H. LEIDERMAN, Amer. J. Physiol. 175, 263 (1953).

¹⁷ R. E. ECKEL, C. E. POPE, and J. E. C. NORRIS, Arch. Biochem. Biophys. 52, 293 (1954).

¹⁸ M. K. GAITONDE and D. RICHTER, Biochem. J. 59, 690 (1955); Proc. Roy. Soc. 145, 83 (1956).

¹⁹ M. K. GAITONDE and D. RICHTER, Proc. Roy. Soc. 145, 83 (1956).

of the specific activity of methionine in the nonprotein nitrogen fraction. It is of interest to compare their turnover rates with those found in our experiments. While in our studies, progressively increasing time intervals between injection and killing of the animal resulted in progressively decreasing turnover rates, GAITONDE *et al.*¹⁹ found no similar relationship and calculated a mean half-life for the proteins of 13.7 days $\pm$ 4.1 (s.d.). Although these authors carried out short interval experiments comparable in duration to ours, the relative constancy of the turnover rate found by the use of radioactive methionine makes one suspect that, with intracisternal administration, mainly the turnover of slowly metabolized brain proteins neighbouring the ventricular cavities was measured (see above).

The finding of the different turnover rates for the proteins of brain, liver and muscle depending on the time interval between administration of lysine and sacrifice of the animal gives an indication of the metabolic inhomogeneity of the component proteins. The turnover rate of liver proteins also decreases with increasing experimental period despite the fact that this organ is relatively homogeneous containing about 60% parenchymatous cells. It is, therefore, not surprising that the same phenomenon is observed in an organ such as the brain which is characterized by a heterogeneous cell population and in which the ratios of neuronal to nonneuronal elements vary markedly from one area to another. The complexity is considerable even within a 'homogeneous' part of the brain such as the cortex, in which the proteins of the perikarya, of the dendrites, of the glia, of the axons, and probably also some mucoproteins of the 'groundsubstance', are all present. Each part of the brain contains proteins with high and low turnover rates; their quantitative distribution is probably different from part to part, and from structure to structure.

The growth increment of mouse brain between the 10th day of life and maturity is small. Nevertheless, an indication of the mechanism of growth in brain and other organs as well is given by the observation that in young animals the absence of the slowly metabolized proteins is not associated with an increase in the turnover rate of the 'fast' fraction. The turnover rate of the 'fastest' protein fraction is apparently rapid enough so as not to be changed significantly by the additional contribution due to the net synthesis of protein in the growing organ. A similar observation was made in an investigation of rate of fatty acid synthesis during growth, when no increase in the rate of deuterium incorporation was noted during the period of myelination²⁰.

The incorporation of lysine into the proteins of an organ can be due to intracellular protein metabolism or to synthetic processes associated with the replacement of dead cells. The latter mechanism would imitate a dynamic state in those organs in which the continuous death and replacement of a considerable number of cells is the rule, a situation which does not hold for the neuronal elements of the brain. It is very unlikely that the replacement of glial elements is fast enough to account for the high turnover rate of brain proteins. Thus, the rapid incorporation of lysine and methionine argues strongly for a dynamic state of brain proteins. Of particular interest in studies of protein synthesis and degradation are those fractions which have a particularly fast turnover. The shortest half-life time calculated for proteins of the whole brain was 2.8 days. This is somewhat slower than

the fastest fraction of the liver, which, in the same experiments, had a half-life time of 1.0 days (Table II). These figures give only an indication of the rapidity of the turnover of some protein fractions of the two organs. If we take the values found for the microsomal proteins (which are probably also a mixture of proteins of different turnover rates), we find that the rate of lysine incorporation into this fraction is about 5 times higher than it is into the proteins of the whole homogenate (Table IV). It appears, therefore, that certain protein fractions of the brain are renewed in a matter of hours.

Histochemical and biochemical evidence for the participation of proteins in the functional activity of brain is scarce²¹. The findings reported in this summary raise the significant question as to whether the spectrum of rates of protein metabolism is the secondary expression of the general energy and nutrient metabolism of this organ, or whether its variety and range contribute to a physiological basis for the functional capacity of the central nervous system.

Zusammenfassung

Mit Hilfe von isotopem Lysin wurde der Austausch von freiem Lysin zwischen Blut und Gehirn, Leber und Muskel und die Umsatzrate der Eiweisskörper dieser Organe bestimmt.

Im Zusammenhang mit den erhobenen Befunden wird die Funktion der Blut-Hirnschranke und die Erneuerung der freien Aminosäuren und der Proteine im Gehirn und in anderen Organen erörtert.

²¹ H. HYDEN, *Acta physiol. Scand.* 6, Suppl. 17 (1943). - L. G. ABOOD and A. GEIGER, *Amer. J. Physiol.* 182, 557 (1954). - R. VREBA, *Physiol. Bohemosloven.* 4, 397 (1955).

COGITATIONES

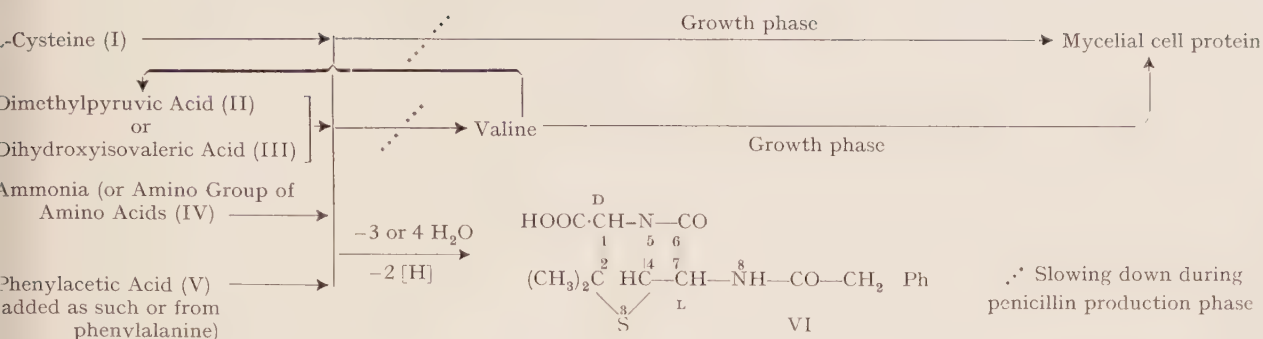
The Biosynthesis of Penicillin

By K. GANAPATHI*

A theory of biosynthesis of penicillin should define: (i) the immediate precursors partaking in the biosynthesis; (ii) the way the precursor units react mediated by enzyme systems to yield penicillin; (iii) the pathways of synthesis of these precursors themselves from the media constituents; and (iv) the specific differences in biochemical terms between the poor penicillin-producing strains of *Penicillium chrysogenum* and the high yielding ones. The knowledge available at present is far from complete. An attempt is made here to marshal the data available and evolve a coherent picture which can form the basis for further work. The significant facts that make the studies difficult are: (1) the biosynthesis of penicillin by *P. chrysogenum* appears to be of no special significance in the economy of the mould itself; (2) in the penicillin-producing phase, the mould synthesises only about 0.01 to 0.03% of its weight of penicillin per hour; (3) even under the best of conditions, the quantity of penicillin produced is only 1 to 5% of the major media chemical used; and (4) the precursors that take part in penicillin biosynthesis are used extensively for other synthetic activities, such as the formation of cellular proteins.

²⁰ H. WAELSCH, W. M. SPERRY, and V. A. STOYANOFF, *J. biol. Chem.* 140, 885 (1941).

* Antibiotics Research Centre, Hindustan Antibiotics (Private) Ltd., Pimpri, Poona District (India).



General Mechanism and Immediate Precursor Units

After critically examining the published work and the actual plant practices, the author formulated¹ as a working hypothesis the following course of events as occurring in the biosynthesis of benzyl penicillin (VI) by the mould from the immediate precursors (I, II or III, IV and V).

It is apparent that penicillin biosynthesis is connected, directly or indirectly, with the metabolism of amino acids as has also been observed by others in a general way². In penicillin fermentation, we have (i) an early growth phase when there is a rapid multiplication of the mycelium, involving the synthesis, among other things, of the entire cellular protein material with very little production of penicillin; and (ii) the penicillin-producing phase, when the multiplication of the mould takes place only to a limited extent³. In the latter phase, there would be some synthesis, though at a much reduced rate, of fresh cellular material and the decay of some mycelia already present; in addition, a dynamic exchange of the amino acids from the metabolic pool with those of the cellular proteins is also probably taking place since there is an incorporation into cell proteins of added labelled amino acids after the growth phase, and also a change in the nitrogen content of the mycelium in the course of time. But the need for the amino acids in this second phase for cell protein synthesis would be much less than in the growth phase and hence the appropriate ones would be available for penicillin synthesis.

That L-cysteine (I) (reduction product of L-cystine) is a direct precursor and is incorporated intact into penicillin (as atoms 3, 4, 7, 6, 8 in VI) has been firmly established by the outstanding work of ARNSTEIN *et al.*⁴ and by STEVENS *et al.*⁵ The studies on the incorporation of labelled D-, L- and DL-valine added to the medium into the penicillin molecule have shown⁶ that (i) the carbon skeleton of valine is incorporated intact; (ii) the amino group of valine is not incorporated; and (iii) L-valine is used at a faster rate than the D-form. Since the configuration at C₁ of penicillin is D, it is very probable that the non-nitrogenous precursor of valine, dimethylpyruvic

acid (II) or the closely related dihydroxyisovaleric acid (III), is the other immediate precursor for penicillin synthesis. That phenylacetic acid (V) is also an immediate precursor has been definitely proved⁷. There remains then only the nitrogen atom 5 to be accounted for. No experimental data is available indicating the source of this nitrogen atom. But, on analogy with the biosynthetic mechanisms involved in the biosynthesis of the purines⁸, we can suggest as possible precursors either ammonia as such, or more probably the amino group donating amino acids, such as glutamine, asparagine, glutamic acid or aspartic acid. If glutamic acid, arginine, histidine, etc. directly stimulate penicillin as reported⁹, then these amino acids may presumably act by donating the nitrogen atom 5.

Penicillin appears to be synthesized within the cells and then excreted out into the metabolic fluid. So the immediate precursors will also be synthesized within, or in case they are available in the medium itself, they should get an entry into the cell, and then be made use of for the synthesis of penicillin.

Interaction of Precursor Units

Without being specific about the exact sequence of the reactions for which there are a number of possibilities, penicillin biosynthesis from the four precursors as defined above can be postulated to proceed as indicated p. 174.

The configuration D at C₁ of penicillin is probably formed when the cyclisation takes place. The oxidative (dehydrogenation) cyclisation in the last step may be a simplified version of what exactly may be taking place. The peptide and amide formations may conceivably involve the participation of peptidases, coenzyme A, and ATP¹⁰. ROLINSON¹¹, after studying the action of various inhibitors of enzymes in the metabolism of *P. chrysogenum*, has concluded, without specifying the exact steps involved that penicillin biosynthesis includes the utilization of phosphate bond energy and also a cyanide sensitive enzyme (involving an oxidation) for the synthesis to proceed on at a maximum rate.

Availability or Synthesis of the Immediate Precursors

Of the immediate precursors, phenylacetic acid is actually added to the medium as such, because the ex-

¹ K. GANAPATHI, Antibiotic Symposium (Pimpri), March 28 1956) (under publication).

² F. T. WOLF, Arch. Biochem. 16, 143 (1948). — P. L. N. RAO and R. VENKATARAMAN, Exper. 8, 350 (1952). — A. J. H. PYLE, J. gen. Microbiol. 11, 191 (1954).

³ H. KOFFLER, R. L. EMERSON, D. PERLMAN, and R. H. BURRIS, *J. Bact.* 50, 517 (1945).

⁴ H. R. V. ARNSTEIN and P. T. GRANT, *Biochem. J.* **57**, 353 (1954); *Biochem. J.* **57**, 360 (1954).

⁵ C. M. STEVENS, E. INAMINE, and C. W. DE LONG, *J. biol. Chem.* **19**, 405 (1956). — C. M. STEVENS, P. VOHRA, and C. W. DE LONG, *J. biol. Chem.* **211**, 297 (1954).

⁶ H. R. V. ARNSTEIN and M. E. CLUBB, *Biochem. J.* **60**, XXXIV (1955). — R. PRATT and J. DUFRENOY, *J. Antibiotics* (Lippincott) **9**, 91.

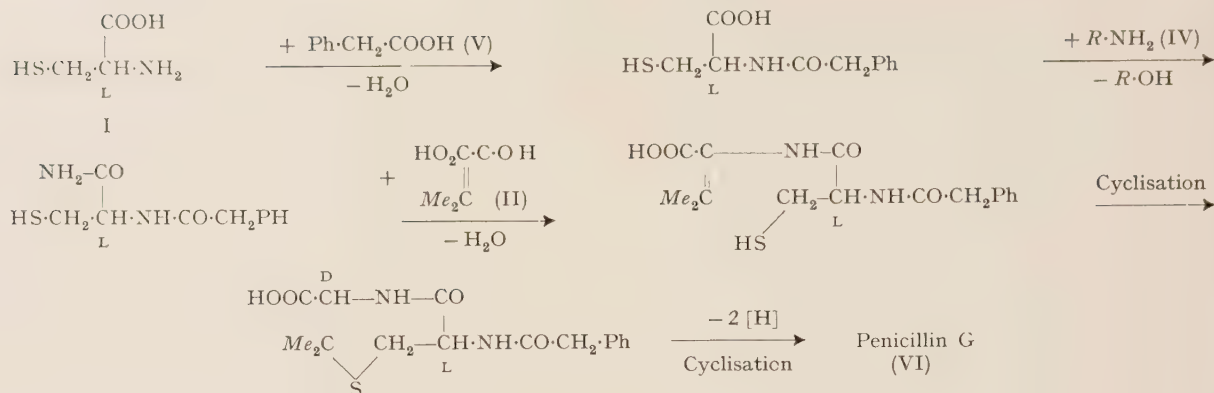
⁷ O. K. BEHRENS, J. CORSE, R. G. JONES, E. C. KLEIDERER, Q. F. SOPER, F. R. VAN ABEELE, L. M. LARSON, J. C. SYLVESTER, W. J. HAINES, and H. E. CARTER, *J. biol. Chem.* **175**, 765 (1948). — O. K. SEBEK, *Proc. Soc. exp. Biol. Med.* **84**, 770 (1953). — M. GORDON, S. C. PAN, A. VIRGONA, and P. NUMEROF, *Science* **118**, 43 (1953).

⁸ B. LEVENBERG, S. C. HARTMAN, and J. M. BUCHANAN, Fed. Proc. 14, 243 (1955). - J. C. SONNE, I. LIN, and J. M. BUCHANAN, J. biol. Chem. 220, 369 (1956).

⁹ J. T. WOLF, Arch. Biochem. 16, 143 (1948). – A. G. C. WHITE, L. O. KRAMPITZ, and C. H. WERKMAN, Arch. Biochem. 8, 303 (1945).

¹⁰ G. EHRENSVÄRD, *Ann. Rev. Biochem.* 24, 294 (1955).

¹¹ G. N. ROBINSON, J. gen. Microbiol. 11, 412 (1954).



tent of conversion of phenylalanine to phenylacetic acid by the mould cannot meet the needs of penicillin synthesis by the high yielding strains. Of the media constituents, corn steep liquor or the proteins which get hydrolyzed to the various amino acids by the proteolytic enzymes of the mould, are ready sources of L-cysteine; but the exact quantities available thus ready-made depend upon the composition of the media used. On the other hand, in a synthetic medium, with nitrate or ammonium ions as the nitrogen source, with lactose, glucose, lactate or acetate as the carbon source, and sulphate as the sulphur source, cysteine, as also the various other amino acids, are actually synthesized by the mould. Even when corn steep liquor or protein containing medium is used, one cannot rule out the possibility of synthesis of these amino acids by the mould. The following compounds have been reported to be utilized for the synthesis of L-cysteine by *P. chrysogenum*: formate¹², glycine¹³, serine¹⁴, cystathionine¹⁴, methionine¹⁵ and sulphate¹⁶. Although our knowledge of the mechanism of formation and metabolism of the amino acids, even where intensively studied¹⁷, is still in a nebulous state suggesting many possibilities, the following course of synthesis of L-cysteine in *P. chrysogenum* appears to be very probable.

It may not be that the entire sequence has to be gone through; the mould may use up whatever intermediate is available from the medium at the time of synthesis. The formation of glycine and formate themselves is still in question at present for lack of precise information.

¹² E. MARTIN, J. BERKY, C. GODZESKI, P. MILLER, J. TOME, and R. W. STONE, *J. biol. Chem.* **203**, 239 (1953).

¹³ H. R. V. ARNSTEIN and P. T. GRANT, *Biochem. J.* **57**, 353 (1954).

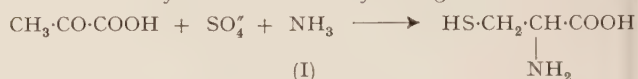
¹⁴ C. M. STEVENS, P. VOHRA, J. E. MOORE, and C. W. DE LONG, *J. biol. Chem.* **210**, 713 (1954).

¹⁵ C. M. STEVENS, P. VOHRA, E. INAMINE, and O. A. ROHOLT, JR., *J. biol. Chem.* **205**, 1001 (1953).

¹⁶ M. GORDON, P. NUMEROF, and S. C. PAN, *J. Amer. chem. Soc.* **76**, 4037 (1954). - E. LESTER-SMITH and D. J. D. HOCKENHULL, *J. applied Chem.* **2**, 287 (1952).

¹⁷ G. EHRENSVÄRD, *Ann. Rev. Biochem.* **24**, 275 (1955).

ARNSTEIN¹⁸ has also visualized a possibility of formation of L-cysteine from pyruvic acid, sulphate and ammonia, which is a very attractive theory owing



to what follows in the sequel. It is not unlikely that, for penicillin fermentation, L-cysteine is made available by more than one way—directly as such from the proteins and corn steep liquor, and by synthesis from carbon, nitrogen and sulphur sources. Dimethylpyruvic acid (or dihydroxyisovaleric acid) may become available either from valine (of the protein or corn steep of the medium) by deamination or transamination, or by the synthesis from a carbon source, via pyruvic acid. That the first possibility exists, has been shown by the incorporation of the carbon skeleton of added valine into penicillin molecule. JOHNSON¹⁹ has shown in his experimental fermentations that a continuous feed of glucose gives rise to high titres of penicillin. From the fact that during the penicillin-formation phase there is utilization of lactose or glucose, it is very likely that these precursor acids II or III arise from the carbohydrates to a major extent. It has been shown in a very convincing way in the case of *Torula utilis*²⁰, *Aerobacter aerogenes*²¹ and *Neurospora crassa*²² that dimethylpyruvic acid or dihydroxyisovaleric acid²³, which serve as the precursors of valine, arise from pyruvic acid by the condensation with its decarboxylated product, acetaldehyde. The entire course of formation of the precursor acids from the basic carbohydrates and also the other metabolites could be formulated as follows:

¹⁸ H. R. V. ARNSTEIN and P. T. GRANT, *Biochem. J.* **57**, 360 (1954).

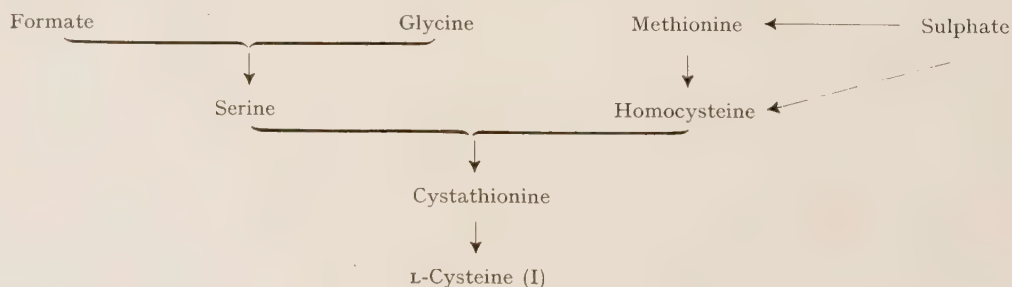
¹⁹ F. V. SOLTERO and M. J. JOHNSON, *Applied Microbiol.* **2**, 41 (1954).

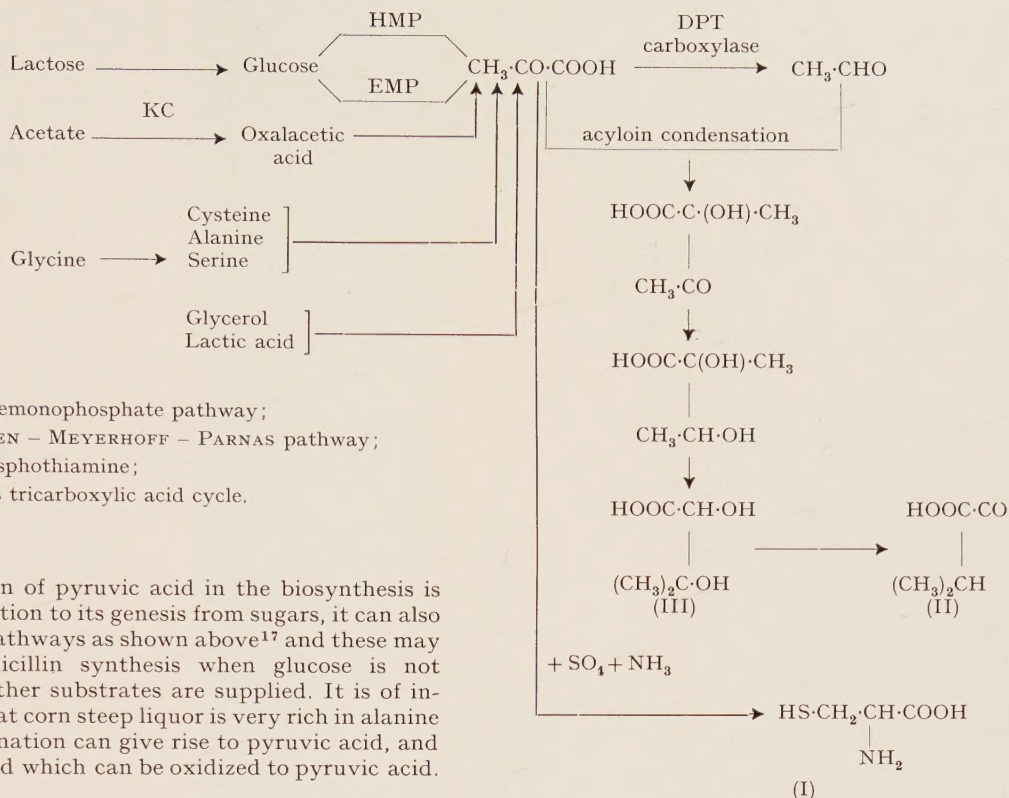
²⁰ M. STRASSMAN, A. J. THOMAS, and S. WEINHOUSE, *J. Amer. chem. Soc.* **77**, 1261 (1955).

²¹ M. E. RAFELSON, *J. Amer. chem. Soc.* **77**, 4679 (1955).

²² B. S. STRAUSS, *J. gen. Microbiol.* **14**, 494 (1956).

²³ E. A. ADELBERG and E. L. TATUM, *Arch. Biochem.* **29**, 235 (1950). - E. A. ADELBERG, *J. Amer. chem. Soc.* **76**, 4241 (1954).





The key position of pyruvic acid in the biosynthesis is evident. In addition to its genesis from sugars, it can also arise by other pathways as shown above¹⁷ and these may operate in penicillin synthesis when glucose is not available and other substrates are supplied. It is of interest to note that corn steep liquor is very rich in alanine which by deamination can give rise to pyruvic acid, and also in lactic acid which can be oxidized to pyruvic acid.

Penicillin Production by Many Moulds and Strains

Although penicillin possesses a unique structure, it is not a unique product of mould metabolism. Almost all strains of *Penicillium notatum* or *chrysogenum*, 25 *Penicillia*, 7 *Aspergilli*, and a few more unrelated moulds, produce penicillin, although the nature of the penicillins vary and the quantities produced are low. The fact that the precursors we have identified are quite common ones met with in any amino acid metabolic pool, explains the fairly wide occurrence of penicillin in the metabolic fluids of moulds. The factor restricting the synthesis of penicillin to the microorganisms mentioned above may variously be, the non-availability of the appropriate precursors at the moment of synthesis, the absence of the enzyme system in the microorganisms that effect the condensation of the precursor units, availability of other pathways which proceed with greater degree of ease, and the failure to obtain conditions that are optimal for penicillin synthesis, such as the neutral pH or low rate of multiplication of the mould.

Coming down to the narrow range of *P. chrysogenum*, the differentiation in specific biochemical terms of the high yielding strains evolved from the wild strains by a series of steps involving treatment with mutagenic agents and selection, from the wild type poor penicillin-producing ancestors, is an intriguing problem. As contrasted with the genetic block in the synthetic steps leading to the accumulation of new compounds in the well-known cases of neurospora and some bacterial mutants, what we meet with in the case of *P. chrysogenum* is the more intense synthesis of a compound already being produced by the parent strain. BACKUS and STAUFFER²⁴, in reviewing their entire strain selection program leading to the development of strains of superior penicillin-producing power, conclude that 'a more or less progressive decline in the vegetative and reproductive vigour of the strains has accompanied the

increase in capacity to produce the antibiotics'; this may be interpreted to mean the slowing down of the rate of synthesis of the mould constituents and making available for penicillin synthesis more of the appropriate substrates. This explanation, also propounded by FOSTER²⁵, appears to be the only generally plausible one at present, though the details have to be elucidated.

Zusammenfassung

Auf der Grundlage einschlägiger Veröffentlichungen wird ein Mechanismus für die Biosynthese des Benzylpenicillins angegeben, welcher dessen unmittelbare Vorstufen und ihren Aufbau sowie die zur Bildung des Benzylpenicillins führende Reaktionsfolge festlegt. Danach erscheinen als unmittelbare Vorstufen L-Cystein, Dimethylbrenztraubensäure oder Dihydroxyisovaleriansäure, Ammoniak oder die Aminogruppe von Aminosäuren und Phenylelessigsäure. Die Vereinigung dieser Vorstufen zu Benzylpenicillin vollzieht sich unter Bildung dreier Peptidbindungen und eines mit Dehydrierung verknüpften Ringschlusses. Für den Aufbau des L-Cysteins wird ein Reaktionsschema vorgeschlagen, welches der bei der Biosynthese beobachteten Aufnahme von Ameisensäure, Glycin, Serin, Methionin und Schwefelsäure in das Penicillinmolekül Rechnung trägt. Die Entstehung von Dimethylbrenztraubensäure oder Dihydroxyisovaleriansäure wird auf eine Azyloinkondensation von Brenztraubensäure mit Azetaldehyd und nachträgliche Umlagerung des Reaktionsproduktes zurückgeführt. Die Tatsache, dass die festgestellten Vorstufen allgemein verbreitete Stoffwechselprodukte sind, gibt eine wahrscheinliche Erklärung für die Bildung von Penicillin durch verschiedene Spezies von *Penicillium*, *Aspergillus* und anderen Pilzen. Die noch nicht völlig geklärten biochemischen Unterschiede zwischen

²⁴ M. P. BACKUS and J. F. STAUFFER, *Mycologia* 47, 446 (1955).

²⁵ J. W. FOSTER, *Chemical Activities of Fungi* (Academic Press, New York 1949), p. 246.

Stämmen mit hoher und niedriger Penicillinproduktion lassen sich vielleicht darauf zurückführen, dass bei ersteren grössere Mengen der unmittelbaren Penicillinvorstufen oder ihrer Vorläufer zur Verfügung stehen, welche bei letzteren zur Förderung des Wachstums und der Fortpflanzung der Pilze dienen.

EXPERIENTIA MAIORUM

Hundert Jahre Schweizer-Reagens

Im Jahre 1857 publizierte EDUARD SCHWEIZER¹, Professor für Chemie an der Universität Zürich, eine Notiz in der Vierteljahrsschrift der Naturforschenden Gesellschaft Zürich (Jahrgang 2, 395–398), betitelt: *Das Kupferoxyd-Ammoniak, ein Auflösungsmittel für die Pflanzenfaser*. Darin wird ausgeführt, wie gereinigte Baumwolle in der blauen Flüssigkeit unter Verquellung aufgelöst wird und nachher durch Ansäuerung mit Salzsäure wieder ausgefällt werden kann. Damit war das später technisch für die Herstellung von Kupferseide ausgenützte Verfahren vorgezeichnet. SCHWEIZER war sich der grossen Bedeutung seiner Entdeckung offenbar voll bewusst, denn er liess seine Notiz im gleichen Wortlaut fast gleichzeitig im Journal für Praktische Chemie [72, 109 (1857)] erscheinen, wo indessen ausdrücklich erwähnt ist, dass es sich um einen Nachdruck aus der Zürcher Vierteljahrsschrift handelt.

Wie SCHWEIZER die zelluloselösende Eigenschaft der Kupferoxyd-Ammoniaklösung entdeckt hat, wird nicht näher ausgeführt. Doch darf man annehmen, dass ihm die Lösung seine Papierfilter zerstört hat. Im Vorjahre hatte er im Journal für Praktische Chemie [67, 430 (1856)] *Über das unterschwefelsaure Kupferoxyd-Ammoniak und die ammoniakbasischen Metallsalze überhaupt* berichtet, wobei er ausführte, wie sich schwefelsaures Kupferoxyd-Ammoniak mit Hilfe von unterschwefelsaurem Baryt in unterschwefelsaures Kupferoxyd-Ammoniak umsetzen lasse, welches nach Abfiltrierung des ausgefällten Bariumsulfates durch Abkühlung als kristalliner Niederschlag gewonnen werden könne. «Sämtliche Portionen des auskristallisierten Salzes wurden alsdann auf einem Filter vereinigt zwischen Papier ausgepresst». Hierbei machte sich die zur Diskussion stehende Eigenschaft der Kupferoxyd-Ammoniaklösung offenbar nicht geltend, denn sie wird in der Arbeit (1856) nirgends erwähnt. Erst als SCHWEIZER 1857 versuchte, aus der dunkelblauen Flüssigkeit ammoniakalischer Kupfersalze das Kupferoxyd-Ammoniak zu isolieren und dessen Eigenschaften näher kennenzulernen, wurde, wie er schreibt, «meine ganze Aufmerksamkeit durch eine höchst interessante Eigenschaft jener Flüssigkeit in Anspruch genommen. Dieselbe besitzt nämlich in ausgezeichnetem Grade das Vermögen, bei gewöhnlicher Temperatur Pflanzenfasern aufzulösen. Übergiesst man gereinigte Baumwolle mit der blauen Flüssigkeit, so nimmt erstere bald eine gallertartige, schlüpferige Beschaffenheit an, die Fasern gehen auseinander und verschwinden, und nach einigem Durcharbeiten mit einem Glasstabe hat sich das Ganze in eine schleimige Flüssigkeit verwandelt».

SCHWEIZER stellte ferner fest, dass nicht nur Zellulose, sondern auch Seide und Kollagen (Tierische Blase) von

Kupferoxyd-Ammoniak aufgelöst und durch Ansäuerung wieder gefällt werden können, während Stärke zu seiner Verwunderung nur zu einem blauen Kleister aufquoll. Damit war lange vor der Entdeckung der Wasserstoffbindungen und verzweigter Makromoleküle bereits aufgezeigt, dass Kupferoxyd-Ammoniak bei den untersuchten Objekten wohl Wasserstoffbrücken, jedoch keine kovalenten Bindungen zu sprengen vermag.

Die erste Untersuchung über die Morphologie der Quellung und Auflösung von Pflanzenfasern ist noch im gleichen Jahre durch CARL CRAMER, Dozent (seit 1860 Professor) für Allgemeine Botanik an der Eidgenössischen Technischen Hochschule (damals Polytechnikum) durchgeführt worden. Seine Beobachtungen *Über das Verhalten des Kupferoxydammoniaks zur Pflanzenzellmembran, zu Stärke, Inulin, zum Zellkern und zum Primordialschlauch* wurden am 23. November 1857 in der Zürcher Naturforschenden Gesellschaft vorgetragen und in der Vierteljahrsschrift [Jg. 3, 1 (1858)] publiziert. Er beschreibt in dieser Arbeit ausführlich die schon von SCHWEIZER beobachtete Kugelquellung der Baumwollhaare und erklärt sie richtig durch die Unlöslichkeit der Kutikula dieser Pflanzenhaare. Er entdeckte ferner die Kugelquellung bei Hanf- sowie anderen Bastfasern und schloss auf eine in Kupferoxyd-Ammoniak unlösliche Oberflächenhaut dieser Fasern (heute als Primärwand bezeichnet). Er zeigte, wie diese unlöslichen Häute bei der Quellung zu Ringen zusammengeschoben werden, und bildete diesen Befund einwandfrei ab. Ferner stellte er fest, dass verholzte Fasern nicht aufgelöst werden können.

VON CRAMER stammt auch die noch heute gebräuchliche Abkürzung Cuoxam für das Schweizer-Reagens, das allerdings in neuerer Zeit für die Fasermikroskopie durch das haltbarere Kupfer-Äthylendiamin ersetzt worden ist.

Seit diesen Pionierarbeiten sind Dutzende von Publikationen über die zelluloselösende Eigenschaft des Kupfertetramins in technischen, chemischen und biologischen Zeitschriften erschienen, denn diese Lösungen lieferten die ersten nicht auf dem Umwege über die Nitrierung gewonnenen Kunstfasern aus Zellulose, und in den Händen STAUDINGERS erwies sich die «schleimige» Zelluloselösung als für die viskosimetrische Molekulargewichtsbestimmung der makromolekularen Kettenmoleküle geeignet.

Wenn man bedenkt, welch grosser Nutzen der Zellulosechemie und der Zellulosestechnologie durch die Anwendung der Entdeckung SCHWEIZERS erwachsen ist, erscheint es angebracht, auf die zwei Originalarbeiten über diesen Gegenstand hinzuweisen, die beide vor hundert Jahren in der Vierteljahrsschrift der Naturforschenden Gesellschaft Zürich erschienen sind.

A. FREY-WYSSLING

Institut für Allgemeine Botanik der Eidgenössischen Technischen Hochschule Zürich, den 17. Dezember 1956.

Summary

Cuprammonia as a solvent for cellulose was discovered a hundred years ago by EDUARD SCHWEIZER, Professor of Chemistry at the University of Zurich, and the heterogeneous swelling of plant fibres with this reagent was described in the same year by CARL CRAMER, Professor of Botany at the Swiss Federal Institute of Technology then recently founded. Both discoveries were published in the 'Vierteljahrsschrift der Naturforschenden Gesellschaft Zürich' 1857 and early 1858.

¹ In den Lehrbüchern findet man auch die (unrichtige) Schreibweise SCHWEITZER.



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Von Dr. B. L. VAN DER WAERDEN, Professor an der Universität Zürich. Aus dem Holländischen übersetzt von HELGA HABICHT und mit Zusätzen vom Autor versehen. 488 Seiten mit 180 Figuren. In Ganzleinen gebunden Fr./DM 37.50. Sammlung «Wissenschaft und Kultur», Band 8.

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Der Autor war bestrebt, das Buch einerseits wissenschaftlich exakt, andererseits aber auch leichtfasslich zu machen in dem Sinne, dass jeder, der an einer Mittelschule Mathematik gelernt hat und sich für die Geschichte der Mathematik interessiert, es verstehen kann. – Die Geschichte der Mathematik erscheint im rechten Licht nur, wenn man sie gegen den Hintergrund der allgemeinen Kulturgeschichte betrachtet. Das Leben in den griechischen Städten in der klassischen Zeit ist anders als das Leben an den Fürstenthöfen von Alexandrien und Syrakus, und die Wissenschaft wird vom Leben manchmal entscheidend beeinflusst.

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